ABSORPTION AND SECRETION IN THE DIGESTIVE SYSTEM OF THE LAND ISOPODS.[1]

BY JOHN RAYMOND MURLIN, PH.D.

I. Introduction.

While the tendency at this day is to approach physiological problems chiefly from the experimental and the chemical standpoints, there is yet need of many morphological observations to supplement these more *ultimate* researches. The chemical history of the transformation of foods into tissues has advanced considerably beyond the morphological history, because until recently no means has been at hand for identifying them by morphological methods. Such determination has been begun by Fischer (1)[2] in his studies on the effects of fixing fluids on various proteid bodies and their derivatives.

Proteid foods have often been traced to the intestinal absorbing cells, and have been identified in the blood of many animals after having traversed the cells; but their course in passing through the intestinal epithelium and their relation to the various cell constituents have been little known. With the hope of throwing some

[1] Contribution from the Zoological Laboratory of the University of Pennsylvania.

[2] Numerals in parenthesis refer to bibliographical list at the end of the paper.

light on these and kindred problems, the present study was undertaken at the suggestion of Prof. E. G. Conklin, in the Zoological Laboratory of the University of Pennsylvania.

It is a pleasure to express at the outset my gratitude to Prof. Conklin, both for turning over to me a subject on which he had made many observations and for rendering great assistance by suggestion and direction throughout the course of the work.

II. Material and Methods.

Several features combine to make the digestive organs of the land isopods especially favorable for a study of this kind. The digestive glands are simple tubes made up of a single-layered epithelium, which is bathed by the cœlomic fluid. From this the cells derive directly the substances elaborated into the ferments; and the secretion discharged at their luminal surface is poured into the anterior end of the intestine, where it is mixed with the food, partially at least, as it enters. That portion of the intestine which performs an absorptive function likewise possesses a single-layered epithelium composed of very large cells. Without going into details here, it is enough to say that the size of the cells is equaled, sof ar as is known to the writer, by those of a similar absorbing organ of only one other animal, the larva of *Ptychoptera contaminata* (2). In a word, the plan of organization which is shared by the digestive system of all Arthropoda, has here been carried out with diagrammatic simplicity.

Whether we regard the organization of the intestine as an adaptation to the mode of life or the feeding habits as an adaptation to the organization, there is plainly a very nice relation between the two. Microscopical examination of the intestinal contents shows that in proportion to the quantity of digestible matter a very large part is wholly indigestible. Bits of dead leaves, wood fibres and various other masses of thick-walled vegetable cells, some clearer, thin-walled cells, which I take for hyphal cells of fungi, are among the most commonly observed substances.[3] In addition there are in the intestine numerous crystalline bodies, doubtless of an inorganic nature, the skeletal remains of insects, and micro-organisms. But the

[3] I have frequently seen pill-bugs eating edible mushrooms and have observed that they shun poisonous species.

scanty proteid content of such food is compensated for by the very efficient absorbing surface.

Judged by the number of pellets of waste, the quantity of food eaten seems prodigious. An animal kept in a clean dish on a piece of moist bark will cast from twenty to fifty in twenty-four hours. The number is about the same for all the species. Brought fresh from the natural state and deprived of food the number cast in a single night (about fifteen hours) averages, for a large number of counts, between five and six; in the next twenty-four hours the average number decreases to three. (This decrease is explained by the fact that only the anterior half of the "mid-gut" is provided with strong musculature. In the absence of muscles strong enough to empty the posterior portion, and with no fresh food to crowd back the remainder, its progress is very much slower; some of it, indeed, may lodge just anterior to the sphincter for as long as two or three weeks, where it may be recognized as a little black mass in the region under the first abdominal segments.) The anterior portion is emptied, as can be seen by holding the animal up to the light, during the first night. Since then five or six pellets represent the contents of the anterior half of the "mid-gut," the animal must eat, in the course of twenty-four hours, an amount which fills the intestine from two to four times.

The several indigenous species of terrestrial isopods are equally favorable. Those which I have used are *Porcellio spinicornis*, *Porcellio scaber*, *Oniscus asellus*, *Philoscia vittata* and *Cilisticus convexus*.

A reserve stock of animals, renewed from time to time, has been kept in the University Vivarium surrounded by the natural objects among which they were found—bits of bark, dead leaves, etc. Placed in large glass evaporating dishes, covered with a glass plate, on the under side of which was kept moist filter-paper for preserving the proper humidity of the atmosphere, the animals behaved in every respect as in the natural state. In the feeding experiments, to be described later, it was necessary first to empty the intestine of all food. The method commonly employed for this was merely to isolate animals in dishes covered as above described and cleaned once or twice daily. No difficulty was experienced in keeping the animals alive in this way so long as starvation was not too prolonged, provided only the atmosphere was kept moist by daily

renewal of water in the filter-paper, and care was taken to leave no large drops of water in the bottom of the dish.[4]

Several observers have remarked the prolonged starvation which the isopods can withstand (Ide (3), Conklin (4), McMurrich (5), Schönichen (6)). No attempt has been made here to determine the ultra-maximum time; but in the course of the study an isolated animal has frequently been starved for more than a month. When several are starved together in the same dish, the time of absolute deprivation seldom reaches a month; some are sure to moult, or to become greatly weakened, or to die from some cause, when the legs and soft parts are then eaten by the others. While this does not necessarily terminate the fast for all, it does make impossible that determination of the fasting period which is often desirable. The only way to obtain this datum with certainty is to isolate the animals. For this purpose small flat-bottomed vessels of various dimensions, preferably all of the same height, so that they could be covered with the same sheet of glass and filter-paper, have been employed.

Various methods of removing the intestine have been tried.

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[4] The latter precaution was necessary to guard against drowning. When by chance the breathing appendages are covered with water, if the animal is in its natural habitat or on filter-paper or other object which will take up the water readily, it very rapidly frees itself by dipping the abdomen, just as one might remove an extra drop of ink from a fountain pen by touching it to an absorbing surface. Kept on clean glass, however, the drop is not always removed, and the animal may die from suffocation or over-exertion, or both.

A greater difficulty was experienced in cases of prolonged starvation from cannibalism. Although in the main vegetable feeders, or scavengers at most, and particularly inoffensive toward other species of animals, these Crustacea will eat one another, if driven by hunger to this extremity. While some signs of offensive attack have been seen, such as a vigorous nip by one animal at the dorsum of another, plainly sufficient to inflict considerable pain, it would be unsafe to regard an act of this kind as a deliberate attempt to kill. Moreover, very young animals have been kept alive in the same dishes with adults through a month or six weeks of starvation. Individuals isolated in small stender dishes have often been seen to moult and to survive the ordeal just as in the state of nature. But if one moult (Sec. iii, (4)) in a dish with other starving animals or is greatly weakened by its efforts to remove a drop of water, it may fall an easy victim to the hunger of its starving companions. The cannibalism to be guarded against is, therefore, of an accidental sort, and arises from an instinct to keep the intestine full. The same instinct prompts the animal in similar circumstances to eat anything it can swallow, although it is not intended by this to imply that no choice is exercised in the selection of its natural food. Mention will be made in a later section of the care necessary to induce animals which have been fasting a long time to eat the pure foods with which it is desirable to feed them.

Dissecting under fluids in the ordinary manner is not satisfactory because of the small size of the animals. McMurrich's (5) method of placing the animal in the killing fluid, and with a needle in each end pulling him asunder; or that employed by Conklin (4) of first removing the head, and then pulling out the intestine by catching the tail segment with a pair of forceps, is much more rapid. In either case the intestine breaks at the junction of the anterior portion, or stomach, with the middle portion, or "mid-gut." For obtaining the intestine and the glands with their connection preserved, the posterior one or two segments are cut off with scissors and the organs removed by pulling on the one or two anterior segments with forceps. The whole mass is easily handled, and the normal relation is preserved through all the fluids. I have usually dissected off the harder chitinous parts of the head in oil just before embedding.

It has been necessary to give special attention to the means and methods of fixation. As will appear more clearly later, the form and relation of the cellular constituents depend upon the direction of penetration of the killing fluid, as well as upon the strength of the fluid employed. By the usual method of plunging the organs directly into the killing fluid the penetration is, of course, from the cœlomic toward the luminal side of the cell. For the purpose of obtaining penetration from the luminal toward the cœlomic side of the intestinal cells an hypodermic syringe was filled with killing fluid, and the needle, made blunt by turning back the point, was inserted through the mouth into the anterior portion of the mid-gut. Then the posterior segment having been cut off as before to free the posterior attachment of the intestine, all the segments back of the second thoracic were slipped off together by holding the anterior end with one pair of forceps and pulling with another. In a few cases I was able to obtain a fairly good injection by inserting the needle after extracting the intestine; but since it always suffers more or less from handling, and it is always difficult to insert a needle into a collapsed intestine without tearing it, the former proved the safer if the more cruel method.

The following killing fluids have been used: *a*, ninety-five per cent. alcohol; *b*, saturated aqueous solution of corrosive sublimate; *c*, the same with addition of two per cent. acetic acid; *d*, picroacetic after Lee, and *e*, after Conklin; *f*, Zenker's fluid; *g*, picro-formalin; *h*, formo-alcohol; *i*, osmic acetic; *j* osmic-bichromate (Altmann's);

k, Flemming's; *l*, Hermann's, and *m*, Hermann's without acetic (one per cent. platinic-chloride 15 parts, one per cent. osmic acid 4 parts). For minute cytological details I have relied most on *a*, *k*, *l*, *m*, because these are most faithful to the structure in fresh cells. Further consideration of the effects of fixation on the different cell constituents and the metabolic contents will be taken up under the appropriate heads.

For studying the structure in fresh cells, the organs were mounted in the blood of other animals of the same species, as follows: Upon a slide a small rectangular fragment of cover-slip was supported by a fragment of glass of suitable thickness, the other end resting on the slide. The blood was now squeezed out of a decapitated animal in the form of a large drop, which, applied to the edge of the cover-glass, was drawn under by capillarity. From one to two dozen animals, according to the size, are required to furnish sufficient blood for a single intestine. The medium having been thus prepared, the organs were drawn out of the animal, placed on the slide, and arranged near the supporting glass parallel to the junction of the two fragments. The quantity of blood was then noted by a mark indicating its level on the cover-glass, and this level was maintained by adding distilled water as water was lost by evaporation. The chemical changes accompanying coagulation of the blood do not seem to affect the cells at all within the first half-hour. Post-mortem changes, however, do occur after an hour or two. Consequently no structure as seen by this method has been trusted beyond thirty minutes from the time of preparation.

Most of my observations have been made on serial sections prepared in the usual manner. Both "liver" and intestine were sectioned, stained and mounted on the same slide. The stains upon which most reliance has been placed are iron-hæmatoxylin, Biondi-Heidenhain, Flemming's triple, Hermann's triple and Altmann's acid-fuchsine-picric-alcohol. The effects of these on the various parts will be mentioned from time to time.

III. Description of the Digestive System.

1. *Gross Structure.*

It will be necessary to recall the gross structure of the digestive system, as this can be made out with the naked eye or a dissecting microscope.

1. *The intestine* takes the most direct course possible from the mouth to the anus. Three chief divisions are generally recognized: *the anterior*, consisting of the œsophagus and grinding stomach (*kaumagen, poche malaxatrice*); the *middle* ("mid-gut;" *intestine moyen*), in which three divisions will be recognized, and

the *posterior* or rectum. Only the middle intestine concerns us in this paper. Its anterior portion is of slightly larger diameter than the posterior (fig. 1). The lateral walls are perfectly straight;

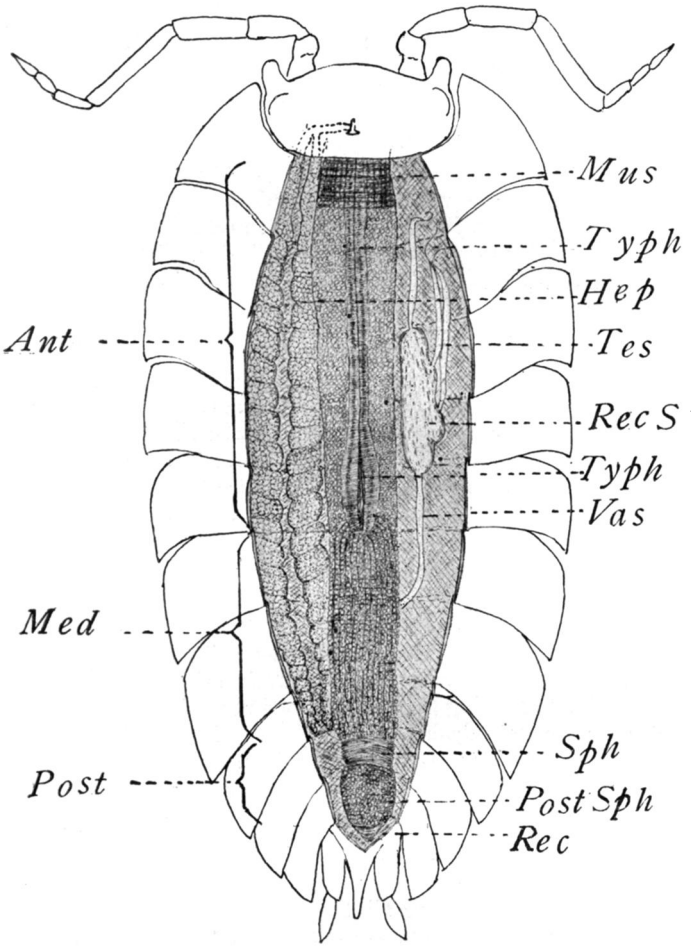

Fig. 1.—*Oniscus asellus* (male, 12 mm.) × 10.—(In this and following figures mm. refers to the length of the animal, measuring from the anterior margin of the head of the segment to the end of the uropods.) Slightly diagrammatic drawing to show relation of the digestive organs. *Ant.*, *Med.*, *Post.*, anterior, median and posterior portions of the "mid-gut"; *Mus.*, muscular coat of intestine, shown only at extreme anterior end of "mid-gut"; *Typh.*, typhlosole; *Hep.*, hepatopancreas; *Tes.*, testis; *Rec. S.*, receptacle of sperm; *Vas.*, vas deferens; *Sph.*, sphincter muscle; *Post Sph.*, post-sphincter; *Rec.*, rectum.

the transition from the anterior larger to the posterior smaller portion is very gradual. The only break in the uniformity of the wall back to the sphincter is a dorsal longitudinal furrow, bounded by two slightly projecting ridges, which extends from the extreme anterior end of the "mid-gut" to a point opposite the junction of the fifth and sixth thoracic segments. At the anterior end of its posterior third the structure thus presented widens out so that, as Ide (3) says, "it terminates like a spatula." The furrow is due to an infolding of the epithelium along the mid-dorsal line (fig. 3), which then spreads out laterally within the lumen, fitting into or covering the secondary grooves formed by the projecting ridges. Following Conklin (see Sec. vi), I shall call the entire structure, grooves and ridges, the *typhlosole*. The "mid-gut" is uniform in size from the posterior end of the typhlosole to the region of the fourth abdominal segment. Here it is considerably constricted by a strong sphincter muscle pinching off, so to speak, a small posterior portion of the "mid-gut," which has already been spoken of as the post-sphincter, and which moulds the pellets of waste before they are ejected. The typhlosole and sphincter mark off three convenient subdivisions of the "mid-gut": the *anterior*, containing the typhlosole and reaching to its posterior end; the *median*, from the end of the typhlosole to the sphincter muscle, and the *posterior*, including that portion covered by the sphincter, and the post-sphincter portion as far as the rectum.

2. *Glands.*—Huet (7) has described for *Ligia* a pair of salivary glands lying on the posterior ventral wall of the œsophagus and opening by an extremely narrow aperture into this portion of the canal. He has demonstrated that they are salivary glands by dissecting them out in alcohol (which does not destroy diastase) and placing potato starch in a watery extract. Dextrose was detected after twenty-four hours. Ide (3) considers these glands as cutaneous appendages, disputing their connection with the œsophagus, although admitting that they *may* be concerned in digestion. On account of their very small size and the uncertainty of their morphological nature, no attention has been given them in this study.

The only other digestive gland with which we have to do in the isopods is the *hepatopancreas*. It consists, in the forms studied, of two pairs of blind tubes, placed a pair on each side of the intestine, into which they open, at the anterior end, by a ⊥-shaped

aperture. The two tubes on the same side unite into a transverse canal which joins that of the other side at the aperture. The tubes extend almost the entire length of the body cavity, their tapering blind ends reaching into the abdominal segments (fig. 1). It is to Weber (37) that we owe the recognition of the mixed nature of this gland and the application to it of the name *hepato-pancreas*, introduced by Krukenburg (24) for the analogous organ of fishes.

2. *Microscopic Structure of Intestine.*

The wall of the "mid-gut," as of the other divisions of the intestine, is made up of four coats which have been recognized by all the later writers. Beginning with the outer or cœlomic side, these are the *muscular coat*, the *basement membrane*, the *epithelium* and the *intima* or chitinous lining. The muscular coat has been fully described by Ide (3) and Schönichen (6). It consists of two layers, an outer longitudinal and an inner circular. Over the anterior portion of the "mid-gut" (*i. e.*, as far back as the typhlosole extends) the outer is imposed upon the inner; posterior to this both layers thin out so that the fibres are quite widely separated from one another, each one running in the groove between adjacent rows of cells. They also fuse together and anastomose freely, so that a muscular network is formed, through the meshes of which the cells project. The sphincter, according to Ide, is an additional layer lying outside the longitudinal and constituting a second circular one. The inner circular layer is very much reduced at this place, so that only a few fibres appear.

The other coats, as McMurrich has indicated, properly belong to the epithelium. Both the basement membrane and the chitinous lining are formed by the epithelial cells. The former is a smooth membrane of uniform thickness which accommodates itself to all the irregularities of the outer surface, dipping into the dorsal furrow and keeping closely applied to the cells. We have already mentioned the very large size of the epithelial cells. As Ide observed, they are often to be seen with the naked eye. They are represented in fig. 1 for *Oniscus asellus*, 12 mm. long, magnified just ten diameters. Those in the median portion of the "mid-gut" are most easily seen, because only partly covered with muscle. Their ends, projecting freely into the cœlome, appear as little mounds on the

contour line. Another striking feature of the epithelium is the rectangular arrangement of the cells in longitudinal and transverse rows. One exception occurs at the posterior end of the typhlosole, at which point the longitudinal rows converge, so as to form, as Schönichen says, "parallel parabolas, making a picture in optical section not unlike a longitudinal section through a vegetative point." While the rows elsewhere are rectilinear, they are not often continuous from one end of the "mid-gut" to the other. I have often seen longitudinal rows which terminate some distance from the end. Discontinuous transverse rows have also been seen. Ide's fig. 19 shows these features, which are supported further by his statement that only the median two ventral rows can be followed uninterruptedly from the anterior to the posterior end.

(1) *Growth of Intestine.*

At first sight the number of rows of cells appears definite and constant enough that one might name the cells as city blocks are named, and might even find cell homology between different individuals. This character has been mentioned by all the authors since Lereboullet (8) (Huet, Ide, Conklin, McMurrich, Schönichen), and McMurrich has attempted to show that the number is constant throughout the life of an individual. McMurrich's attempt is based on the non-occurrence of cell division. No observer except Lee (9), who regarded certain deformed nuclei stretching from one cell to another as remains of the spindle (*fuseau de regrèssion*), has seen any sign of mitosis, and McMurrich says: "I have never been able to satisfy myself that normal amitosis actually occurs." He seemed to be justified, therefore, in the attempt (which by his own confession is not wholly successful) to show that growth of the intestine takes place by enlargement of the cells *rather than* by multiplication.

Amitosis.—In young individuals amitosis is by no means rare. Fig. 2, *A*, *B* and *C*, show some of the stages which are easily found at this time. Fig. 2, *A*, is a longitudinal section exhibiting the relative sizes of cells whose nuclei have just divided, and of those recently constricted off. Conklin (*l. c.*) has rightly interpreted this constriction of the cells, and has figured what he regards as an amitotic division. It would appear from fig. *A* and fig. *B* (which is a horizontal section showing division in the transverse direction) that the nuclei may completely divide and move some distance apart before constriction of the cytoplasm takes place. From one or two observations made on very young porcellios in process of moulting, I am led to think that the constriction may even be

postponed until the next moult of the intestinal lining. The old intima evidently would present considerable resistance to the formation of a furrow, which is always to be seen in the intima between

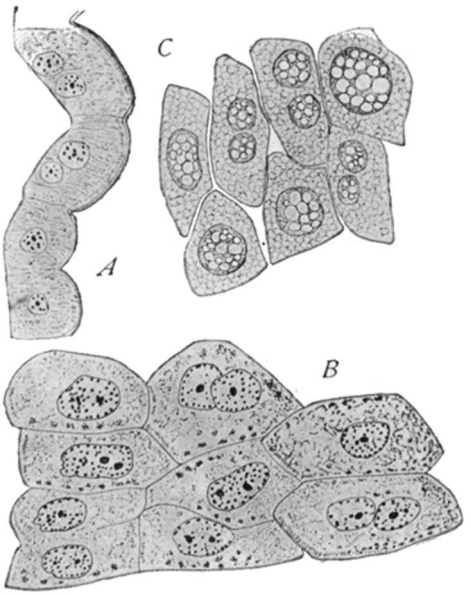

Fig. 2.—*A*, longitudinal section of intestine of a young *Porcellio* (3 mm.); *B*, transverse section of the same; *C*, optical section of intestinal cells of young *Porcellio* (1.8 mm.) immediately after moult of the posterior half. All these figures illustrate growth of the intestine by amitosis. *A* and *B* × 280, *C* × 600, nuclei in *C* show post-mortem changes.

cells. Moreover, there is nothing to indicate that the nuclei represented as already separate in these figures (*A* and *B*) have not been so for a considerable time, while the frequent occurrence of two nucleated cells, with no other sign of division, would speak strongly for this.

Several authors have reported cells with two or three nuclei, and Carnoy (10) finds as many as ten to thirty nuclei in the intestinal cells of *Cirolana*. So large a number is due, I have abundant reason to believe, to fragmentation of the nuclei, not to amitosis, as Carnoy states (*l. c.*). Schönichen discredits McMurrich's evidence (to the same effect as my own), holding that the fragmented nuclei resolve on focusing carefully into the nuclei of the muscle fibres. He evidently has not seen the phenomenon reported by McMurrich. Space does not permit an account of the unquestionable cases of fragmentation which I have seen. A whole plate of figures might

be given of cells containing from a dozen to twenty-five fragments of the nucleus. No explanation of the phenomenon can be given at present further than to say that there are many reasons to regard it as pathological, or "degenerative" in Löwit's sense (see page 296).

I have found the intestine of a young *Porcellio*, 1.8 mm. in length, which had recently moulted the posterior half (Sec. iii, (4)), to be literally filled with amitotic division, both longitudinal and transverse, throughout its length (fig. 2, *C*). Many nuclei were in process of division; those already separated were approximately half the volume of others not yet beginning to constrict. There can be no doubt that the large number of divisions was correlated with the increase in size of the intestine immediately following the moult. It seems equally plain that two nucleated cells which are larger than adjacent ones, as in fig. 2, *A*, may represent precocious nuclear divisions, the constriction which will divide the cell being deferred to the next moult, when the luminal end of the cell will be free to cover the new furrow, as well as the old surface, with chitin.

It is evident that amitosis has been seen in the isopod intestine by a number of observers. Carnoy (10) claims to have seen "the constriction of the nucleus in the epithelium of the intestine of the Crustacea, *e. g.*, *Oniscus asellus, Ligia, Armadillo asellus, Idotea, Cirolana*, etc." He figures amitosis in the testicle cells of the isopod, and says these represent exactly what is to be seen in the nuclei of intestinal cells, so far as the phenomena of division are concerned. It is possible, however, that Carnoy, as McMurrich and Schönichen think, may have mistaken distortions of the nucleus for amitosis. Indeed, v. Bambeke (11) refers to one of the figures given by Carnoy as a deformed nucleus. Ziegler and vom Rath (12) say that the "mid-gut" of *Oniscus, Porcellio, Cymothoa* and *Anilocra* show amitotic nuclear divisions with special clearness and very abundantly ("besonders deutlich und recht häufig"); but as will be seen under the subject of *the nucleus* later, and as was recognized by Schönichen, it appears probable that they, too, confused with amitosis certain distortions of the nucleus. Conklin says, speaking of elongated nuclei extending into two cells, "some of these, I am convinced, are amitotic divisions of the nucleus." Since these observations have been called in question by McMurrich on the basis of the very frequent misjudgment of irregularly shaped nuclei, it has seemed worth while communicating my own observations. In conclusion, I may say there is no possible doubt that amitosis does actually occur in the growing intestine, nor any doubt that it has here the same significance which Frenzel (13) ascribes to the phenomenon in the mid-gut of *Astacus* and other decapods, namely, a true cell multiplication ("nicht einzig und allein eine Kernvermehrung, sondern ebensowohl auch eine wahre Zellvermehrung," p. 559).

In other words, it is a " regenerative " as distinguished from a degenerative process. Löwit (14) distinguishes between these two in the following: " Die erste führt nach meine Auffassung zur Neubildung von Kern und Zelle, die letztere kann mit sekretorischen und assimilatorischen Vorgangen im Zellleibe zusammenhängen und durfte wahrscheinlich in vielen Fallen der ausdruck eines bevorstehenden Kern und Zelltodes sein."

(2) *Origin of the Typhlosole.*—In specimens of *Porcellio spinicornis* 1.8–2 mm. long I have been able to follow the origin of the typhlosole. This structure, as we have seen, is but an infolding of the mid-dorsal wall of the " mid-gut," accompanied by a secondary

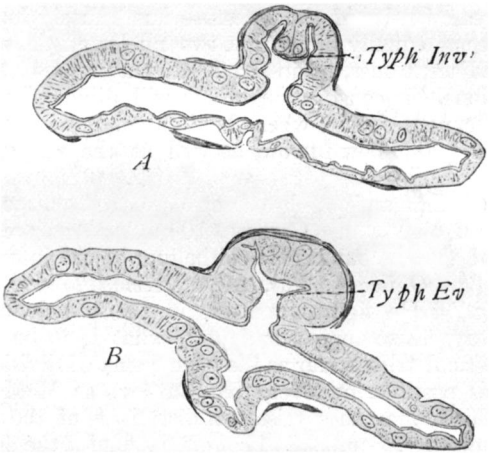

Fig. 3.—Transverse sections of empty intestine of a young *Porcellio* (1.8 mm.). × 180. *A*. At extreme anterior end of the "mid-gut"; *B*. Sixth section (.04 mm.) posterior to *A*.; *Typh.Ev*. Primary evagination in formation of the typhlosole; *Typh.Inv*. Secondary invagination of median two rows of cells which form the middle portion of the typhlosole.

outfolding on either side which appears as a ridge; but it is so definite in form as to be plainly a specialization as well, and my observations show that its development is a progressive one. Cross sections of the anterior portion of the " mid-gut " in animals just hatched show a perfectly uniform unfolded wall, the typhlosole not yet to be seen. In fig. 3, *A*, of a specimen 1.8 mm. long (taken in February and therefore probably several months old), the section is from the extreme anterior end; the next (fig. 3, *B*) is only six sections (about .04 mm.) farther back. The latter shows the earlier stage. The mid-dorsal portion of the wall is bulged out over a considerable extent of the circumference. The cells composing the bulged portion are very open in structure, like

those found in the extreme posterior end of the adult typhlosole. They are larger in all dimensions than those in the rest of the wall, so that the effect is such as would be produced by swelling. Since I have found the structure identically the same in five different individuals of the same age, killed in three different fluids, it seems certain that the swelling is normal and due to some intrinsic growth energy. Fig. 3, *A*, shows that the evaginated or bulged portion on the dorsal side is but preliminary to an invagination of the median two cells whereby the mature form of the typhlosole is approached. These cells have only to grow farther into the lumen and to expand laterally, so as to cover or fill the inner grooves formed at the sides, in order to attain the adult form. The invagination may involve more than these two cells; the stalks separate at their bases later, as shown in fig. 4, *A*, so that the division between these and the next cells must be purely arbitrary.

In the anterior portion the cells rapidly recover from the swelling which is so plain a feature at the first evagination (figs. 3 and 4), while in the posterior portion they retain this loose structure permanently (fig. 4). Here also the middle cells are not turned

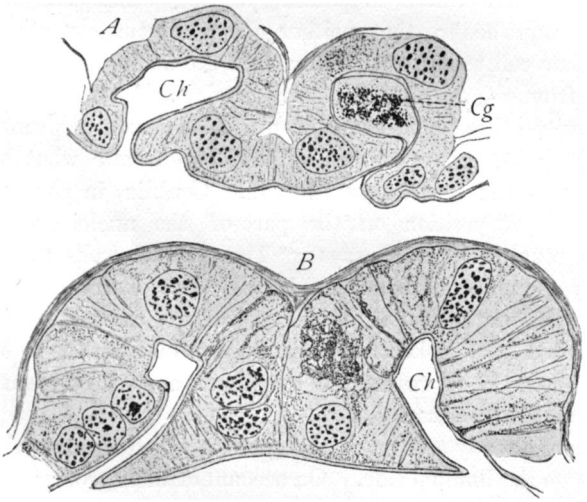

Fig. 4.—Transverse sections intestine of young *Oniscus* (4.4 mm) × 270: *A*, through anterior portion of the typhlosole; *B*, through posterior end of the typhlosole, same magnification; *Ch.*, channel for passage of secretion or liquid food; *Cg.*, coagulum of secretion of hepatopancreas. *B* shows the effects of killing fluids on the large cells of the typhlosole.

in so far, while the lateral extension at their luminal border is rather wider than it is farther anterior. The posterior portion of which we have been speaking is the spatula-like enlargement men-

tioned on page 291). It will appear evident from figs. 4, *A* and *B*, that six rows of cells, counting by the nuclei, are involved in the typhlosole.

(3) *Syncytium.*

The very large cells which compose the adult "mid-gut" were recognized by Huet to be without distinct parietal walls, but to be bounded by rows of fibres. Ide figures cell walls for *Oniscus murarius*, and thinks Huet was in error or overlooked them. Almost simultaneously McMurrich and Schönichen put forward the view that the epithelium is in reality a syncytium, the walls being replaced by fibres which run from the intima to the basement membrane. Both authors show that what Ide saw is undoubtedly the chitinous lining, dipping in between the anterior cells on the luminal side and the basement membrane, which has a similar relation to the cœlomic side of the median cells. The syncytial nature they argue further from the facts that the cells cannot be separated by maceration in potash (a thing which Ide claims to have accomplished); that neither silver nitrate nor other stains differentiate cell walls; and finally, that the nuclei may be squeezed through from one cell into another. Ryder and Pennington (15), who described these movements of nuclei as a natural phenomenon (see page 304), figure cell walls, but do not state what becomes of them when the nuclei pass through. Conklin, in showing that the changes of position on the part of the nuclei are due entirely to rough treatment, says: "The parietal walls are so thin that they cannot be distinguished." My own observations confirm those of the later writers. In the majority of cases of adult intestines where I have looked for them, no sign of a wall is to be seen. Figs. 15 and 16 represent the cytoplasm as perfectly continuous from one cell to another.

We have already spoken of the grooves which separate the anterior cells on the luminal side. On account of these grooves the intercellular fibres are shorter than the cell axes. The same is true for the cells of the median portion and for those of the transitional region, but for a slightly different reason. While the intima does not dip in between the median cells, the latter project into the cœlome at their centres more than at the edges (fig. 12). The cells in the transition region immediately posterior to the typhlosole show an intermediate condition; the intima does not dip in so deeply between

the cells as in the anterior (fig. 21), and the cells do not project so far into the cœlome as in the median portion. The intercellular fibres consequently are again shorter than the cell axis.

Inside the cells, as has often been described, occur other fibres taking the same course from the intima to the basement membrane. Their disposition is varied, sometimes in bundles, as in fig. 4, or singly, as in fig. 9. Again they may be strong throughout their length, as in fig. 4 (usually so in the typhlosole), or may thin out at one end, as in fig. 9. In sections they cannot often be followed continuously from intima to basement membrane because their course is not often straight. They are more frequently straight in the typhlosole where they are thicker than elsewhere (figs. 3 and 4). Where the fibres approach the intima or basement membrane they are parallel and nearly always straight. This gives the so-called "striated" border which has so often been described for the "mid-gut" of isopods (Lereboullet, Leydig (16), Huet, Ide, McMurrich, Conklin, Schönichen). The great regularity of the fibres along the intima is due to a thickening of them for a short distance in, thereby producing what Schönichen calls the "Bälkchenzone." Both McMurrich and Schönichen regard this thickening as due to a deposition of chitin on the fibres, basing their conclusion on a refractive index in the fresh condition, and on a staining reaction in the preserved material, similar to that of the inner border of the intima. Practically all authors agree that the thickened ends are continuous with the intra-cellular fibres. I shall designate this zone of thickened fibres by the term *palisade*, by which I would emphasize merely the parallel arrangement of the fibres at their ends. Very often the spaces between the thickened ends are empty of cytoplasm, which of course strengthens the impression of a separate zone. This zone does not have definite limits, as can be seen in starved cells, where it may extend into the cell as far as the nucleus. In such a case the only difference discernible in the course of the fibres is that they are slightly thicker at the luminal end. Again, as may be seen in figs. 9 and 11, the cytoplasm fills the intervals between the fibres all the way to the intima. Thus the width of the zone may vary from *nil* to more than half the thickness of the cell, according, as we shall see, to the physiological condition of the cell.

McMurrich speaks of the brittleness of the fibres, inferring this property from their cleavage in sections. Conklin calls attention

also to the fact that they sometimes curl at the end when cut, and are therefore elastic. I have seen both of these effects, and would add merely that I have seen them in the intercellular fibres and those of the typhlosole more commonly than elsewhere.

Ide regarded the fibres as mere thickenings of the cytoplasmic reticulum, a view in perfect consistency with the reticular theory to which he holds. McMurrich does not agree that they are mere thickenings, and insists that they are entirely independent of the reticulum. McMurrich is quite right in affirming this independence, as many of my figures will show; nevertheless, it is true, as he discerns, that the fibres are cytoplasmic products. The only exception I would take to his view is with reference to their origin. He states that "in very young specimens of *Porcellio* and *Armadillidium* no trace of the fibres is to be discovered; and in a specimen of *Oniscus* measuring only 4 mm. in length they are but slightly developed, projecting into the cytoplasm from the basement membrane [from which they develop] but a short distance." Unfortunately McMurrich does not state how large his "very young specimens" were. In the youngest specimens of *Porcellio* which I have sectioned (less than 1.8 mm. in length) the fibres are already very evident, although as is to be expected not so strongly developed as in the larger specimens (figs. 2 and 3). I have also sectioned the intestine of an *Oniscus* measuring only 1.8 mm., and find the fibres stretching *all the way through* the cell. I cannot say how early the fibres arise, for these were the youngest individuals to be had in the fall of the year. Unquestionably McMurrich saw fibres which were cut obliquely and which *appeared* not to extend all the way through the cell. The figure to which he refers is evidently from a moulting animal (see fig. 8, page 309). There does not appear to be very good reason for inferring the origin of the fibres *from* the basement membrane merely because they can be traced *to* the membrane. My figs. 11, *B*, and 12 exhibit complete continuity between basement membrane and fibres. A safer conclusion from the facts would be that the fibres and membrane are both formed by the cytoplasm and *may* be formed in continuity. This would account for the appearance of a cell membrane in fig. 12.

a. Cytoplasm.

The earliest observers saw in these cells the fibrillar structure of the cytoplasm only. Leydig (17) states that what he described in former

papers with lower powers as lines ("dicken granulären streifig erscheinenden Zone"), he now makes out to be a "fadiges Balkenwerk dessen einzelne Saulchen ausserdem nicht glattrandig sind sondern seitlichen Zacken haben, durch deren gegenseitige Verbindungen ein zartes netziges Wesen zwischen ihnen entsteht." Ide says, "The partisans of the fibrillar structure of protoplasm as opposed to the reticulated may see here an object very favorable to their thesis" (*l. c.*, p. 158). He recognized, however, as did Leydig, the falsity of such a view, and both authors commit themselves to the reticular structure.

Later writers also have described this structure more or less explicitly. McMurrich says: "The cytoplasm in young specimens has a uniform finely reticulated structure, but this is replaced in the adult by a much less uniform arrangement." Conklin says, in speaking of the disappearance of the nuclear membrane: "The cyto-reticulum is continued into the nucleus," etc. Schönichen finally accepts this structure also, and Hardy (18) figures a "net" structure for the fixed cells of *Oniscus*.

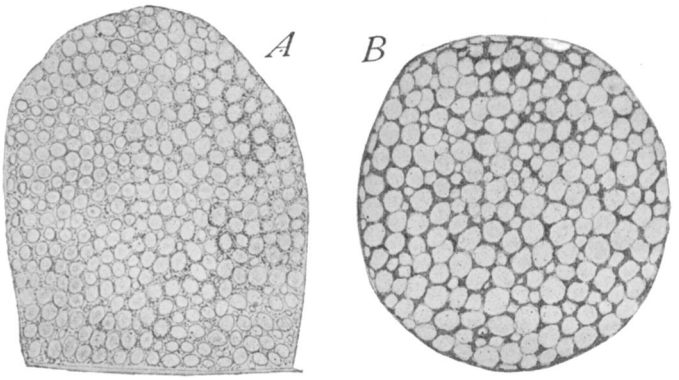

Fig. 5.—Optical sections of: *A*, cytoplasm; and *B*, nucleus of median absorbing cells of *Porcellio*, showing alveolar structure. Drawn with camera lucida from fresh cells, × 925.

None of the authors named seems to have studied the fresh cells. If a fresh intestine be mounted in blood (see page 289), and one attempts to draw the structure as it may then be seen with high powers, he will soon be convinced that the reticular is not the true structure. In all cases I have by this method found the structure to be alveo-

lar (figs. 5, 6, 11).[5] This structure was observed in the fresh cell long before finding a fixation which preserves it. Subsequently,

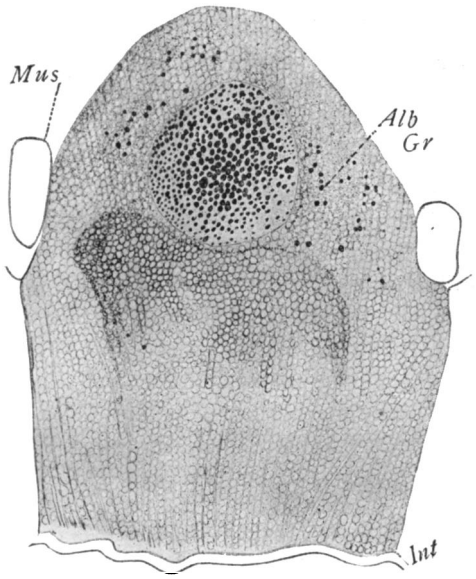

Fig. 6.—Cell from median portion of the "mid-gut" of *Oniscus asellus*, fixed in 1 per cent. platinic chloride 15 parts, 1 per cent. osmic acid 4 parts, sixteen hours after feeding, and stained in iron hæmatoxylin. Drawn with camera lucida, × 600. The alveolar structure of the cytoplasm is preserved. The shaded portion below the nucleus indicates an artifact produced by unilateral penetration of the killing fluid (see page 306). The alternation of fibres and alveoles is shown. *Alb. Gr.*, albumose granules; *Int.*, intima; *Mus.*, muscle.

by examining very carefully with high powers sections from an in-

[5] It is possible that even this structure is due to "sub-mortem" changes, as Hardy indicates. I have made many attempts to discover the alveolar structure in the gut of very young, transparent animals while still living; but without success. Nevertheless it would be premature to conclude from Hardy's studies on artificially prepared colloids alone that there is no formed structure in the living protoplasm. Should this be shown in time, we should then necessarily conclude that the coagulation incident to the "sub-mortem" changes is practically instantaneous, since the structure shown in Fig. 5, can be seen *as soon as* the preparation can be made (within one-half minute from the removal of the intestine). Pending further studies on this subject, I refrain from calling this alveolar structure, living.

It should be said that this paper was in press before Hardy's very suggestive one on "Structure of Cell Protoplasm" was seen.

testine fixed in Hermann's without acetic, I found the alveoles well preserved (figs. 11, *A*, 12, etc.).[6]

Two obstacles appear to stand in the way of recognizing the alveolar structure by ordinary methods. In the first place, the interalveolar substance, concerning which more will be said later, precipitates in the form of granules in the interstices between the alveoles, so as to look much like the nodes of a reticulum. In the second place, the contents of the alveoles precipitate also as fine granules on the alveolar walls. So that after sublimate-acetic fixation, for instance, the effect is precisely what one would expect from a reticular structure. The best conditions, therefore, for observing the true cytoplasmic structure would be where the interalveolar substance is less abundant, and the contents of the alveoles is slightly or not at all precipitated. Fig. 6 represents such a place; Fig. 7, *A*, is also favorable in places (*e. g.*, centre near the top). In the former figure the alveoles are represented by continuous lines as they should be; but for representing the combined effect of alveoles and granular substance, it has been necessary in the interest of simple drawings to adopt the conventional method seen in all the other figures. In fig. 6 it will be seen that the fibres have a very definite relation to the alveoles; often in this intestine, and in many others, the alveoles are plainly between fibres, and the fibres between alveoles as represented. This structure is easily seen in the free ends of the median cells of a fresh intestine mounted in blood. It is easy to understand how Ide and later writers should have mistaken this arrangement for the reticular, and should have supposed that the fibres are joined by trabeculæ. Ide seems to have recognized the independence of the fibres, where he says: "If it were shown that

[6] The fluid was suggested to me by Misses Foote and Strobel (19), who were able to preserve the alveolar structure (hyaline globules) of the eggs of *Allobophora fœtida* in it much better than in any other fluid. Their photographs of the living egg and of the structure as preserved by this fixation, which I have been permitted to see, are very convincing. Use had been made of this fluid to some extent early in the study, but as the nucleus appeared not to be so well preserved as with other fluids, it was set aside temporarily; more recently it was employed throughout a series of physiological stages, with results far more satisfactory for many purposes than were obtained with any other fluid. However, the alveolar structure has been met, occasionally very well preserved, after Hermann's, Flemming's, picro-formalin, and sublimate acetic (fig. 10, *B*). Ordinarily with these and all other fluids, except the platinic-chloride and osmic acid mixture, the structure appears to be reticular.

in certain cells strongly differentiated and old, such as those with which we are dealing, there exist fibres without connections, nothing would stand in the way of admitting that certain trabeculæ break their bonds and are isolated," etc. (*l. c.*, p. 158). The apparent connection of fibres with reticulum is due to bad fixation. Sublimate-acetic, Hermann's, usually Flemming's, and a number of other much trusted fluids produce a similar effect; but it is not the picture one gets in the fresh cell, the structure of which is not difficult to determine.

The alveoles measure on the averagee 2μ in diamter in all ages. Between them in the fresh cell is a substance in which I have not been able with a $\frac{1}{12}$ immersion to recognize granules or any other formed elements. Granules do appear at times about the nucleus, but these have no part in the ground structure. With nearly all fixations, except possibly alcohol and formo-alcohol, the homogeneous interalveolar substance precipitates in the form of fine granules visible with the immersion lens.

b. Nucleus.

aa. Form.—The nucleus of the "mid-gut" cells has been the object of several investigations within the period of modern technique. In 1885 Carnoy probably mistook some unnatural forms of the nucleus for stages of amitosis. In 1887 v. Bambeke described many of these unnatural forms in the "mid-gut" cells of *Oniscus* and other isopods, which may be produced artificially. His method was to examine the intestine in blood of the animals, either with or without the addition of methyl green. For permanent preparations he fixed in osmic acid, stained with methyl green, and mounted in glycerine. He recognized clearly that the irregular forms, frequently found in such preparations, were due to some mechanical disturbance.

Ziegler and vom Rath, like Carnoy, doubtless erred in supposing the elongated nuclei to be in process of division. Ryder and Pennington, overlooking v. Bambeke's paper, described elongated nuclei stretching from one cell to another as a form of non-sexual conjugation of nuclei. All subsequent writers have recognized that for the most part these deformations are due to mechanical causes incident to preparation of the intestine for study. Schimkewitsch (20), in calling attention to this error, says: "Bei Bearbeitung

mit Perenyischer Flussigkeit erhielt ich mit Leichtigkeit alle jene Figuren, welche diese Autoren abbilden." Conklin showed that, by pressing a particular part of the intestinal wall with a pencil point, all the nuclei in this region were caused to stretch out in a radial manner from the place pressed. McMurrich distinguished between irregular forms found after rough treatment, and those which he regarded as natural. Schönichen declares that he never found any but spherical nuclei in well-preserved intestines.

McMurrich and Conklin thought the nucleus might be more or less amœboid in life, like those described by Korschelt (21) for the egg-cell of *Dytiscus*, and that not all the processes found in carefully mounted intestines were abnormal, but might be an index of the physiological condition. The processes stretching toward the lumen

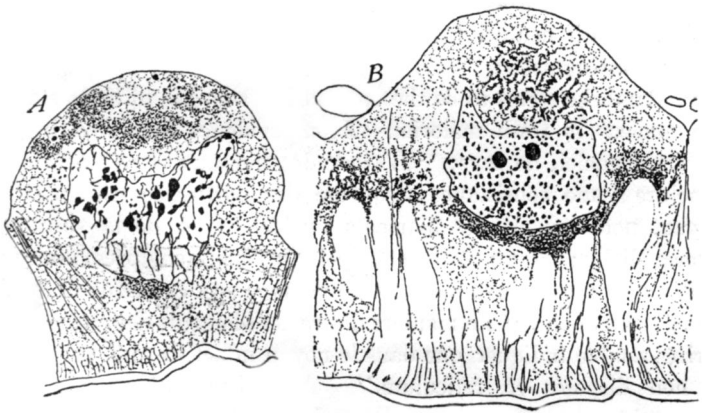

Fig. 7.—Cells × 600 from "mid-gut" of *Porcellio*, showing artifacts produced by injection of fixing fluids into the lumen of the intestine and consequent unilateral penetration by the fluid. *A*, injected with Hermann's fluid; *B*, with picro-acetic (after Bolles Lee). The nuclei exhibit "amœboid" processes.

in fixed preparations were regarded as specializations for receiving food from that direction. For a time this seemed to me to be the state of affairs, and a number of sketches were made representing what seemed to be the escape of nuclear substance also from the processes toward the lumen. Processes of greater or less size were found on this side after all fixations, and it appeared that they must therefore be normal; yet I could not see them in fresh cells of any physiological condition. Finally, at Prof. Conklin's sug-

gestion, several intestines were injected with killing fluid, to see if penetration of fluid from the lumen would cause processes toward the cœlome. The result is shown in fig. 7, *A* and *B*. Nothing could be more convincing. The processes of the nucleus, and in large measure all deviations from the spherical, or at least regularly curvilinear outline, are to be regarded as due to fixation. The latter are the forms found in the living cell, and they are the only ones considered in this paper to be perfectly normal.

bb. Structure.—Several authors have described more or less minutely the structure of the nucleus in fixed material. In adult cells after fixation it is filled with large granules of chromatin, between which are traces of linin. From one to many nucleoli are always more or less distinct. In young cells the chromatin is not so abundant, as McMurrich figures, and as may be seen from figs. 2 and 3. With neutral fixations like formo-alcohol, oxychromatin may be distinguished.

v. Bambeke seems to be the only author who has hitherto studied the nucleus in fresh material. From evidence furnished by the distorted nuclei, he says: "L'étude des noyeaux étrirés permet de conclure à une consistance visqueuse des parties constituantes du noyau, notammuent des filaments nucléolaire et de la substance intermèdiaire; les nucléoles (nucléoles plasmatique) présentent une consistance plus forte et resistent d'avantage aux causes de déformation;" and again, "La manière d'être des filaments dans les noyaux étrirés semble indiquer que dans le noyau intact ils sont pelotonnés et non disposés en reticulum." All other authors who have observed the deformed nuclei have drawn similar conclusions with reference to its consistency.

As may be observed from fig. 5, I have found the structure of the fresh nucleus plainly alveolar, like that of the cytoplasm, except that the alveoles are larger. The relative size may be seen in figs. 5, *A* and *B*. Both are drawn at the same magnification, the cell in *A* being much smaller than that from which the nucleus in *B* is taken. The alveolar structure may by chance be preserved by over-fixation, *e. g.*, "osmication," to use Bolles Lee's term. In neither the fresh nor this over-fixed condition are granules to be seen; but they are brought out sharply by nearly all fixations when the action is not so powerful. I see no way to account for the over-fixed alveoles on any other hypothesis than that of Fischer, who

regards the chromatin as a more or less complete solution of nuclein bodies, which are precipitated ordinarily in the form of granules by fixing fluids. If the chromatin is already in the form of alveoles, over-fixation might result from too rapid action of the fluid on the periphery of the alveole, preventing a deeper penetration, and consequently preventing complete precipitation in the form of a granule. The "skein-like" arrangement (pelotonée) of the chromatin of v. Bambeke would thus be accounted for. Certainly the "osmicated" nuclei look as if such a partial precipitation had taken place. How to regard the strands which appear in distorted nuclei (Fig. 7, *A*), whether as the mere elongation of alveoles or as due to rupture with escape of chromatin contents, I am wholly undecided. I hope to make this and related subjects the object of a special study.

(4) *Moulting.*

We have now considered the normal cellular structure common to all physiological conditions, except those incident to the shedding and renewal of the lining. Early in the course of this study it became necessary to separate clearly the changes occurring in the cell during these processes from those connected with the phenomena of absorption of foods. It is well known that the land isopods moult frequently during the spring and summer. Kept in the Vivarium and Laboratory at temperatures corresponding to these seasons, the moults take place in a perfectly normal manner even in winter, and may easily be observed. It is well known, too, that the test of the isopod is cast in two pieces. The posterior piece, which comes off first, reaches to the anterior border of the fifth thoracic segment. One or two days may intervene before the anterior piece is shed.[1]

[1] In two cases observed in February, of which I kept careful account, the time was about forty-eight hours from the complete detachment and removal of the posterior half until the animal was freed from the anterior half; in several other cases observed in July the time was twenty-four hours. A number of animals kept in dishes were suspected of eating the posterior test while the mouth-parts were still hard. Quite recently I have obtained indubitable evidence that this occurs in the natural state. In a number of animals found with the anterior half still soft the intestine was filled with a chalky-looking substance, which proved on examination to be finely broken bits of the chitinous skeleton; no entire posterior test was found with these animals. The purpose of this habit is probably to utilize the calcareous matter.

It is said that the lining of the "mid-gut" comes out when the posterior half of the test is shed (Schönichen), and this I have seen in a number of cases. Sometimes, however, the lining is not to be found with the posterior half, but remains in the lumen attached, it may be, at some point. The habit of eating the posterior test (see note 7) may possibly be concerned with detaching the lining in such cases. The lining begins to break loose from the epithelial cells before there is any apparent sign of the external moult. Fig. 8, from an animal in the act of casting its intestinal lining, presents an appearance in striking contrast with that seen in other physiological conditions. The fibres are wholly wanting on the luminal side of the nucleus, and the cytoplasm has a uniformly granular aspect; the alveolar structure is almost entirely masked by the granules, so that only rarely does one get a glimpse of it even with high powers. The palisade at the luminal edge is not to be seen. Schönichen has observed this disappearance and concludes that the chitinized ends of the fibres (Bälkchen) function in breaking loose the intima, in the way the "little hairs" described by Braun (22) are said to do for *Astacus*. He has not, however, noticed the difference between the two cases, namely: that in *Astacus* the little hairs are to be seen in the test both during the shedding and after it has taken place, whereas in the isopods the fibres wholly disappear. The disappearance of the fibres is too complete to be accounted for by the mere masking of the alveolar structure by means of granules. This is in strong contrast with a case of masking which occurs when the end of the cell is filled with small granules, referable to the process of food absorption (fig. 11, *B*). The thickened portion of the fibres does not disappear in such masking; they may even appear more sharply defined than where such granules are not present (fig. 11, *A*). At a later stage in the process of moulting, fibres, very much finer than those which still exist unaffected by the moult in the cœlomic side, can be traced in the luminal side after the new intima has attained some thickness. The fine fibres do not possess the strong affinity for stains which they have later. From the foregoing facts it is only reasonable to suppose that the fibres in the luminal side of the cell contribute to the formation of the substance by whose transformation the new intima is formed; and that their disappearance at the time of moulting is due to some process of dissolution, the fluid thus

formed precipitating in the form of granules which, as we have said, mask the true structure of the cytoplasm. On this hypothesis an explanation is readily afforded for the varying strength of the palisade in different individuals. When very strong—*i. e.*, composed of thick fibres from the intervals between which the cytoplasm has withdrawn—the very evident "striated border" described by so many authors is present. In such cases we may suppose the animal to be approaching a moult. When the palisade is scarcely visible (fig. 11, *A*) a moult has probably occurred shortly before feeding.

In the case of moulting figured (fig. 8) the intestine was taken

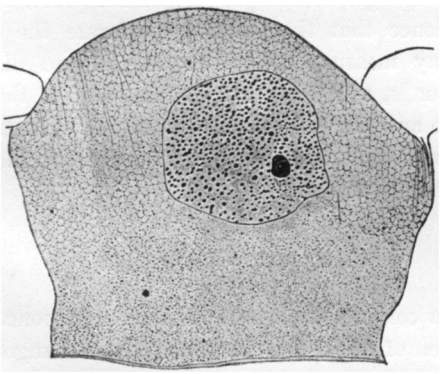

Fig. 8.—Median cell × 600 from intestine of *Oniscus asellus*, fixed in sublimate acetic immediately after moulting the chitinous intima. The lower part of the cell is filled with chitinogenous substance in granular form. All fibres have disappeared from this side of the cell. Intima is very thin.

forty-two hours after feeding. The quantity of food in granular form on the luminal side of the intestinal cell at this time is usually small, and when it does occur the granules can be distinguished from those of the chitinogenous substance. The latter are smaller, nearly uniform in size, equally distributed and very abundant. While I have not been able to trace all the steps in this process, it seems probable that there is here a dissolution of fibres in some points like that described by Mathews (23) for the zymogen of the pancreas of many vertebrates.[8] The new intima is laid down by some process of hardening of the chitinogenous substance.

[8] To any one familiar with Mathews' paper the difference will be obvious. The zymogen granules he derives directly from the fibres, which are traceable in some cases to the chromatin. Granules *may* occur here previous to precipitation by the killing fluids.

While McMurrich declares the intima to be impermeable, both Conklin and Schönichen speak for the existence of pores through which food may pass. These are best seen in the fresh intestine. When the intima is found with the posterior half of the test, one has only to mount it in water to demonstrate clearly the pores in all parts. They are a little more numerous per unit area of surface in the median than in the anterior portion. Owing to the relatively uniform structure of the chitin and its high refractive index, it is difficult to make out more than little pits at the surface, *i. e.*, the luminal end of the pores. In sections made from a fresh intestine with a freezing microtome and mounted in gum-arabic I chanced to get an oblique view of the pores, which supplied the direct evidence that they actually perforate the intima. One can in this way measure both their length and diameter. The former corresponds, of course, to the thickness of the intima, and avarages in the anterior portion for several individuals 1.6μ, in the median portion 2.4μ. The average diameter of the pores for a number of intestines was .5μ.

3. *Summary of Structure.*

We have now considered the complete structure concerned directly in the absorption of food, and have noted the changes in the cells due to the process of moulting. To recapitulate, the apparatus consists of an epithelium of large cells covered by a thin basement membrane, which alone intervenes between the cell body and the cœlome, and lined by a porous layer of chitin. On the mid-dorsal wall of the anterior portion of the epithelium six longitudinal rows of cells participate in the formation of a typhlosole. The cells have no parietal limiting membranes, but are separated quite distinctly from each other by intercellular supporting fibres. The cytoplasm is alveolar. Between the alveoles course the intracellular fibres from the intima to the basement membrane. Each cell contains a large spherical nucleus alveolar in structure in the fresh condition, filled with large granules of chromatin in the "perfectly" fixed condition. At the luminal side of the cell the intracellular fibres are parallel and are thickened so as to form a more or less rigid palisade, from the intervals of which cytoplasm may be excluded. The thickened ends of intracellular fibres serve, firstly, to preserve

an open structure on the free margin of the cells, and, secondly, to furnish some substance essential to the formation of the intima.

IV. Feeding Experiments.

We come now to the main purpose of this paper, namely: to consider the cellular phenomena during the absorption of foods. We shall take up in order the changes to be observed in the cell during the process of absorbing (*a*) proteid and (*b*) fatty foods, reserving for later consideration the changes effected within the lumen by the digestive fluids under the subject of secretion in the hepatopancreas.

It will be necessary to mention briefly the methods of feeding. Animals were starved for various lengths of time, then allowed to eat fresh raw beef for ten minutes, after which they were isolated and killed at successive intervals. Table I (which appears at the end of the paper) is arranged with reference to the number of hours after feeding at which the intestines were fixed. Other foods containing proteid, such as raw and cooked oatmeal, bread, white of egg and other meats, were used for control and comparison, but by far the larger number were fed finely-chopped raw beef. For uniformity only those which were fed on beef are mentioned in the table.[9]

(1) *Effects of Starvation.*

In examining cells after feeding, two factors are to be carefully separated, namely: the effect of previous starvation, and the change produced by the food. It will be necessary now to make mention

[9] Some precautions had to be taken to induce the animals to feed continously. Thus, if they were brought from a dark room directly to a well-lighted one, none would eat unless protected from the light by an opaque object—a chip or bit of black paper. On the other hand, if kept in the light for several days the animal became accustomed to the greater intensity, and would usually eat at once. Even these would eat more contentedly if the food were spread on the under side of an object, as a box-lid, and this placed at an angle so that the animal's body was suspended. Again, if they were allowed to find the food in the course of their wanderings, the chances of eating continuously were improved. Even with these precautions the only way to be sure of the time was to watch the individual and keep note by some mark, such as a spot of asphalt-cement or India-ink on the dorsum. Ten minutes continuous eating was found to be a good average meal of beef. Mention has already been made of the dilute condition in which the land isopod obtains its food. If after long starvation one were allowed to fill the intestine entirely full, the result was sometimes fatal; although after a shorter period of starvation or taken fresh from the natural state, they could be kept alive indefinitely on a constant meat diet.

of the effects of the former. Inspection of fig. 7, *A*, shows that the fluid contents of the cell are free to move from one part of the cell to the other. Some of it is seen here coagulated on the cœlomic side of the nucleus. The alveoles in the lower part of this cell were uncommonly distinct. Comparison of this figure with 9 shows

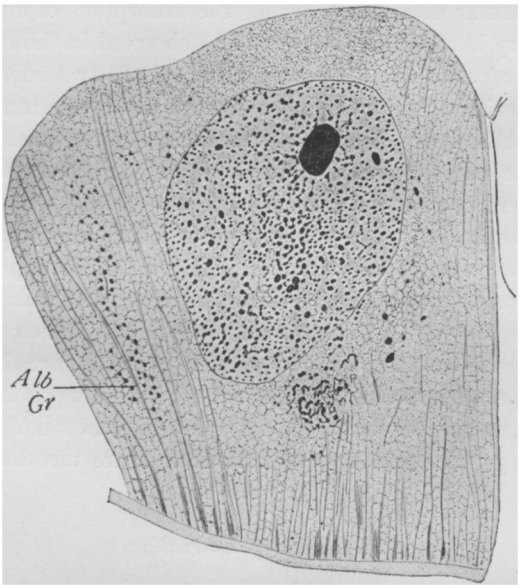

Fig. 9.—Median intestinal absorbing cell × 600 from *Porcellio scaber*, taken from natural environment. Intestine fixed in Hermann's fluid by simultaneous injection and immersion. *Alb. Gr.*, albumose granules.

that the quantity of granular substance is considerably reduced in the former. The former is from an animal starved twenty-four days, the latter from one taken fresh from the natural environment. In the cœlomic end of the median intestinal cells of animals starved a considerable time (*e. g.*, three weeks), one often finds vacuoles. Again, great extension of the spaces which occur in the palisade next the lumen may be seen. These also are confined mainly to the median cells, and the effect is due merely to a withdrawal of cytoplasm from the luminal toward the cœlomic side (fig. 12).

V. Absorption of Proteids.

Let us now examine some cells from intestines fixed at different intervals after eating. Fig. 10, *B*, shows several cells from the

intestine of an animal which had been starved eleven days and then killed four hours after feeding (No. 7 of table). Besides the usual alveolar structure and the interalveolar substance, are to be seen definite aggregations of fine granules. These masses may be at almost any position in the cell, though rarely at an extreme distance from the nucleus. The cell on the extreme right of Fig. 10, *B*, is cut just to one side of the nucleus and in a plane in which the granular substance is specially aggregated—a very common

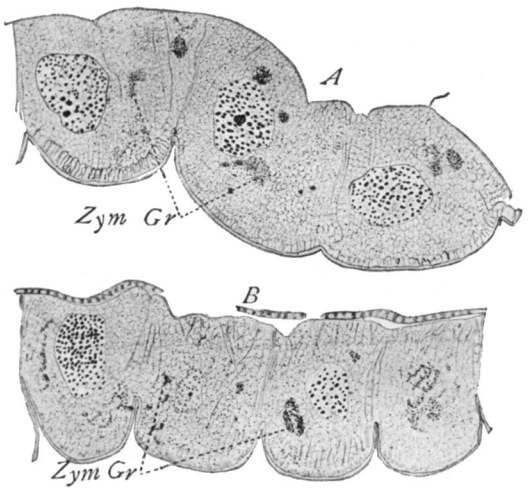

Fig. 10.—Sections × 270 from "mid-gut" of: *A*, *Oniscus asellus*, 16 hours after feeding with raw beef, fixed in Flemming's fluid; *B*, *Porcellio scaber*, 4 hours after feeding with raw beef, fixed in 1 per cent. platinic chloride 15 parts., 1 per cent. osmic acid 4 parts; *Zym. Gr.*, zymogen granules appearing about the nucleus.

arrangement. Often the masses are in actual contact with the nucleus. When the nuclei are broken or shrunken, granules indistinguishable from these are found about the nucleus or opposite the break. There seems good reason, therefore, to state that this substance is being given out by the nucleus. I cannot satisfy myself, however, that the nuclear membrane disappears or that the nucleus suffers any visible change in the process. Fig. 10, *A* (No. 20 of table), shows aggregations which are decidedly more definite. Figs. 11, *A* and *B* (Nos. 8 and 21 of table),[10] exhibit some variations in the form of these masses. All the figures thus far referred to are

[10] Tables I and II follow at end of paper.

of the anterior cells. In the column of the table devoted to these cells, wherever "small granules in masses" are mentioned, reference is made to the substance here spoken of. It will be observed from this column also that in all fixations, except those which blacken the cell contents, the granules are yellowish or brownish even after staining. With the osmic fixations they are always black. After such fixations they take the stains used. The median cells have not, up to sixteen hours after feeding, undergone visible change.

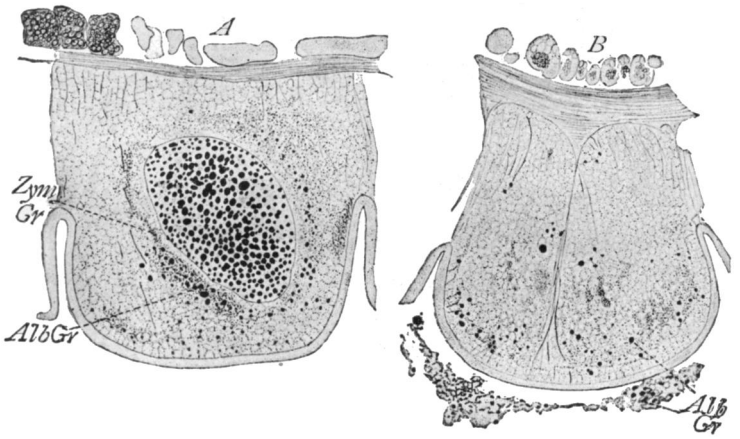

Fig. 11.—Cells × 600 from anterior portion of "mid-gut" of *Porcellio scaber*: *A*, 8 hours after feeding; *B*, 18 hours after feeding, both fixed in 1 per cent. platinic chloride 15 parts, 1 per cent. osmic acid 4 parts. *Alb. Gr.*, albumose granules; *Zym. Gr.*, zymogen granules.

In figs. 11, *A* and *B*, are seen numerous other larger and deeper staining granules scattered in the finely granular substance of which we have just been speaking. In fig. *A* particularly they seem to be quite definitely aggregated with reference to this substance. The small granules come from the nucleus, and there can be no doubt as to the origin of the large ones. Fig. *B* contains the same kind of granules in the lumen of the intestine, mixed with the coagulum which represents the secretion of the hepatopancreas. Since the food is almost pure proteid, and we know from the researches of Huet that the secretion of the "liver" is capable of changing proteid to "peptone" (albumose), we may presume that some product in this process may be found here. Fischer's researches

on the effects of different killing fluids on proteid bodies enable us to decide this question with a high degree of probability. He classes albumoses among the "Granulabildner"—that is to say, they are precipitated in the form of granules insoluble in water by tannin, chromic acid, sublimate, platinic-chloride, formaldehyde, osmic-acetic, Flemming's and Hermann's mixtures; and they are precipitated in the form of granules soluble in water by alcohol, acetone, picric acid, picric acid-alcohol, picric-sulphuric acid (*loc. cit.*, p. 33). The fixation method of determining the presence of albumose would then be: (*a*) Precipitation with some one of each of the two classes of fixatives (Fischer recommends osmic-acetic and Hermann's for the first, and alcohol and picric acid for the second); (*b*) washing both in water; (*c*) staining with some dye which has a strong affinity for albumose (Fischer recommends Altmann's acid-fuchsine-picric-alcohol). If albumose is present it will be found in the first case, and will be washed out in the second. Figs. 1 and 2, Plate XVI, are from the same region of two intestines fixed twenty-four hours after feeding, the former in Hermann's fluid, the latter in alcohol; both were washed a long time in water (the former twenty-four hours, the latter fifteen); they were carried through the same reagents together, and finally stained in precisely the same manner, namely, in 15 per cent. acid-fuchsine in aniline water for five minutes at 54° C., then differentiated in picric-alcohol. (The effect of the picric-alcohol cannot be shown in the figure.) Figs. 3 and 4, Plate XVI, are from intestines treated as above after fifty hours from time of feeding.

In order to test Fischer's results more fully, I have performed a number of experiments similar to his on proteids obtained directly from Grübler's Laboratorium in Dresden. A summary of these experiments is given in Table II. It will be seen that my results confirm Fischer's in most respects essential to the precipitation of albumose. There is but one noteworthy difference, namely, that whereas Fischer obtained from Grübler's hemialbumose (protalbumose) a granular precipitate essentially like that from deuteroalbumose, I find only coagulum by $HgCl_2$.[11]

Peptone (depur. sicc. aus Fibrine, Grübler) seems to be a mixture of albumoses and true peptones. It is altogether likely that

[11] Several other fluids gave the same.

such a mixture occurs in the cells during absorption. I was unable to get a precipitate of the true peptones, after the removal of albumoses by $(NH_4)_2SO_4$, even by the strongest fluids ($HgCl_2$, Flemming's fluid, 1 per cent. chromic acid), which also is in agreement with Fischer. We may safely conclude that the large granules above mentioned contain albumose, and as such they will be designated hereafter.[12]

The exact nature of the finely granular substance, previously mentioned as coming from the nucleus, may now be considered. The very strong evidence that it is derived from the nucleus, and the fact that it is associated with albumose granules in the cell, both speak for a substance elaborated for the purpose of acting on the food. Fig. 14, sixteen hours after feeding, from a fresh intestine stained fifteen minutes in dilute Bismarck-brown, shows masses of granules about the nucleus. The appearance is strongly suggestive of zymogenesis (see figs. 20 and 21). Granules are very often seen about the nucleus in fresh intestines not previously stained, such as in fig. 14, and are not therefore to be ascribed to the effect of the fluid used. Since proteids can enter the cell only in the form of solutions, we can scarcely suppose that we are dealing with any stage of the food. If we may speak of the substance tentatively as zymogen, what further evidence have we that it is destined for ferment action ? When the lumen of the intestine contains secretion from the hepatopancreas, as is always the case when this body appears in the cells in large quantity, it is difficult to be certain whether or not these granules are poured out to mix with the secretion. If the quantity in the cell is great it is sometimes found on the luminal side; but the definite aggregations, which occur most often near the nucleus, are not found here, nor does one ever see distinct evidence of a streaming through the intima. Now, considering that inter-alveolar substance, with which this is to be classed, is capable of being moved by the killing fluids, and that the penetration in the cases mentioned is toward the lumen, it should be found on that side in greater quantity if it is destined for action in the lumen. Notwithstanding this evidence, however, and the further fact that, although it is plainly different from the "liver" zymogen, no secretion different from that of the "liver" can be distinguished

[12] It will be understood, of course, that the granule is in reality a compound of albumose with the precipitating fluid.

in the lumen, I would not go so far as to say that it *may* not escape from the cell. The structure of the cell plainly admits this possibility. All we can say at present is that where we might expect evidence of its passage into the lumen, no evidence is obtained.

To sum up the evidence thus far: The granules as such come from the nucleus; they are associated with food (albumose) in the cytoplasm; they stain only with difficulty; finally, there is no clear evidence that they escape from the cell. All these indicate an intracellular ferment. If it is such we should expect that it would appear in the cell before the food does, or even before feeding. Fig. 10, *B*, four hours after feeding, contains no albumose; it rarely appears in the cell earlier than the eighth hour after feeding. In the table, No. 3 is one of several intestines from animals starved two weeks and not fed before killing; small granules were found in considerable number which do not take the Biondi-Heidenhain stain. Again, McMurrich reports for starved specimens of *Armadillidium* some yellowish granules (which he regards as disintegration products apparently identical with these). Finally, if it is an intracellular ferment it should be found in *all* absorbing cells. The definite aggregations of granules which are found in the anterior cells are seldom met with in the cells of the median part. However, No. 44 of the table, killed in sublimate, stained in Biondi-Heidenhain, exhibited in these cells "small poorly staining granules on the cœlomic side" (also Nos. 13 and 18). In neither of these cases are the granules in masses; they are scattered all through the cœlomic end of the cell. While, therefore, the granules are abundant and definitely aggregated in all stages of absorption only in the anterior cells (see Table I), they do occur in all absorbing cells. There is evidence, which I shall consider presently, that some of the small granules of feeble staining capacity may represent a stage in the transformation of the food itself.

From eight hours onward to one hundred and twenty hours after feeding, albumose granules may be found in greater or less quantity in all the cells. The amount met with in any particular cell will depend upon the accidents of its formation in the lumen. The course it takes, once in the cell, seems to have no distinct reference to the organization of the cell, except so far as that may influence it mechanically. Fig. 2, Plate XVI, twenty-four hours, and Fig. 9, fresh, show the granules arranged in rows, following in a general

way the course of the fibres. In these cases the albumose is plainly not influenced by the nucleus. If absorption goes on continually for some time a mass of granules will be found after fixing, on the cœlomic side of the nucleus (Figs. 12, sixteen hours, and 4, Plate XVI, fifty hours).

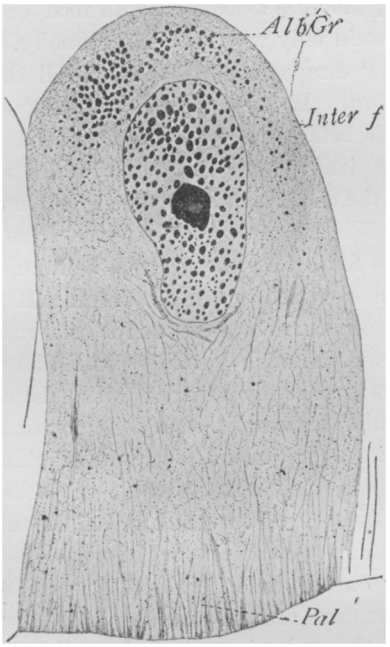

Fig. 12.—Absorbing cell × 600 from median portion of "mid-gut" of *Oniscus asellus*, 16 hours after feeding, fixed with Hermann's fluid. Intima is torn off. *Alb. Gr.*, albumose granules; *Pal.*, palisade; *Inter. f.*, intercellular fibre, showing continuity with the basement membrane.

It has been shown by Fischer (*l. c.*, p. 36 *et seq.*) that the size of the granules of albumose depends upon the strength of the solution, and upon the strength—*i.e.*, precipitating power—of the killing fluid. Thus he finds the average diameter of the granules precipitated by Altmann's osmic-bichromate mixture from a 10 per cent. solution is 1μ to 3μ; from a 3 per cent. solution, $.7\mu$ to 1.5μ; from 1 per cent., $.5\mu$ to $.7\mu$. Again, the average diameter from a 10 per cent. solution precipitated by Flemming's fluid is $.7\mu$ to 1μ; by Hermann's, $.7\mu$ (fairly uniform); by 1 per cent. platinic-chloride, $.7\mu$ to 1μ; by 7 per cent. sublimate, $.4\mu$ to 1μ and *smaller*. Those represented

in figs. 8 and 9, Plate XVI, and in many other cells of the same intestine, measure from .5μ to 2μ; they were precipitated with picro-acetic.[13] The granules in fig. 12 and several other cells of the same intestine measure from .5μ to 1.5μ; they were precipitated with Hermann's fluid. It is not important to attempt an exact determination of the strength in which the albumose may exist in the cells; but the above figures indicate that it may be as strong as a 10 per cent. solution, making allowance for the fact that Fischer's measurements were made for pure solutions, whereas in the cells they are of course mixed with other interalveolar substances. Fischer notes that albumose in mixture with an albumen precipitates larger granules than from pure solutions. He also observes that precipitated from such mixtures they are not uniformly distributed through the coagulum of the albumen, but are collected into small and large nests ("Anhaufung in Nestern"). Figs. 8 and 9, Plate XVI, and others show this arrangement.

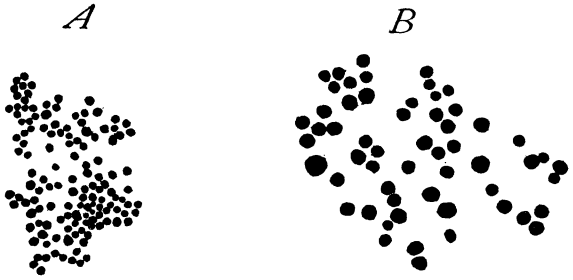

Fig. 13.—Granules of Peptone (depur. sicc. aus Fibrine), (Grübler) × 925. *A*, 2.5 per cent.; *B*, 5 per cent. solution precipitated on cover-glasses by Chromic acid (1 per cent.), stained in Iron-Hæmatoxylin. See Table II, Nos. (5) and (6).

The occurrence of little spaces about each granule of albumose in a coagulum of albumen, which Fischer has figured (*l. c.* Taf., figs. 2, 3, 4), is confirmed also by my figs. 6, 8 and 9, Plate XVI. This fact enables one to determine with clearness the relation of the granule to the alveolar structure of the cytoplasm. Thus, if the granule were precipitated *within* the alveole, and the structure were

[13] Picro-acetic is said by Fischer to precipitate albumose in a soluble form. I find this to be true if the fixation is followed by washing with water, but if followed with 70 per cent. alcohol the granules are not dissolved in this or subsequent fluids. The same is true of picro-sulphuric.

not destroyed by the fixation, the space should correspond to the neighboring alveoles in size—*i. e.*, in such cells as that represented in fig. 6, where the alveoles are so evident, the granule would be plainly seen encased by the alveolar wall. Such an appearance is seldom met with—indeed, the granule is often larger than the alveole (figs. 9, 11, *B*, 9, Plate XVI). Again, in favorable places (figs. 6, 8, Plate XVI), the alveolar walls can be seen intersecting the space about the granule.[14] The space does not correspond, therefore, to an alveole, but represents the area in the interalveolar substance influenced by the killing fluid to form a single granule. It appears, therefore, that the soluble food is independent of the cytoplasmic structure.

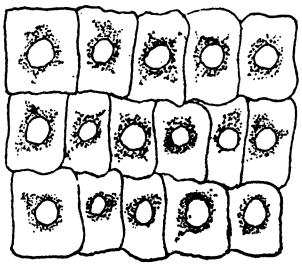

Fig. 14.—Outline drawing (with camera × 90) of an optical section of anterior cells, "mid-gut" of *Oniscus asellus*, fed with raw beef; intestine mounted in blood and stained 15 minutes in dilute Bismarck brown (1: 20,000) 16 hrs. after feeding. The granules about the nucleus take the stain and appear to be coming from the nucleus. This is precisely the appearance of zymogenesis in the hepatopancreas when treated in the same manner (figs. 20 and 21).

Having distinguished the albumose granules from the rest of the cell contents, we may inquire whether further chemical stages of the food appear in the fixed and stained sections in a form distinguishable by the microscope. It was mentioned farther back (p. 317) that evidence might be found for regarding some small granules of feeble staining capacity as a stage in the transformation of food. In the first place, it must be stated that the digestion of the food (raw beef) is pancreatic (*i. e.*, alkaline[15]) (see Sec. ix, *a*); and

[14] Cf. Hardy's figures, *loc. cit.*

[15] This we have on strong comparative evidence also. Thus Plateau (24) demonstrated (*a*) that the reaction in the crop of the Carabidæ and Dytiscidæ may be neutral before feeding, but is always alkaline after; (*b*) that the reaction in the "mid-gut" of the carnivorous chilopod *Lithobius* is alkaline, in that of the herbivorous diplopod *Julus may* be acid.

according to Neumeister's (27) scheme, the first bodies chemically recognizable in the tryptic digestion of proteids are deutero-albumoses. Now, deutero-albumoses, according to Fischer and this study, behave as we have described under the general name albumose (see p. 315). If granules appear before albumose is formed, therefore, it is not probable that they represent food. May those in question, however, not be albumose in a weaker solution, seeing that the size of the granules depends upon the strength of the solution ? In answer to this question two facts may be mentioned: (*a*) whereas albumose granules take stains readily, these granules stain with difficulty; (*b*) where only a small number of albumose granules are present they are usually very much larger than these (figs. 8 and 9, Plate XVI). If, then, any of the granules designated as "small and poor-staining" (see Table I) are food, they must represent a stage following albumose. Fischer has found the true peptone (in Kühne's sense) very difficult to precipitate, and I have been unable to get any precipitate at all with killing fluids from the filtrate after treating Grübler's preparations of albumoses with $(NH_4)_2SO_4$. If the true peptone were precipitated *in the cell,* the granules would in all probability be very small, and Fischer finds them also very difficult to stain (wenig tinctionsfähig). Both these properties are exhibited by the granules in question. It is possible, therefore, that some of the small non-stained granules occurring with the albumose, or after albumose may be expected to have been formed, are true peptone. Granting this, however, we should still have to account for *a*, the appearance of the granules of this description in the starving cells, and *b*, the origin of many granules found in the living cells and in the fixed material immediately about the nucleus. The ferment hypothesis is still necessary to account for both these facts.

The attention of the reader will have been arrested by the densely staining mass represented in figs. 6 (text) and 8, Plate XVI, lying on the luminal side of the nucleus and extending toward the lumen. It will be seen to consist of densely staining strands (fig. 8), matted

Hoppe-Seyler (25) found the action of the "liver" ferment of the crayfish *Astacus fluviatilis* to be pancreatic and not peptic, although the reaction was slightly acid. Krukenburg (26) has shown the same to be true also of several Brachyura. Finally, the general adoption of the name hepatopancreas for the digestive gland of the Arthropoda was in recognition of the pancreatic nature of its secretion.

together and enclosing some albumose granules. In fig. 6 it consists, for the most part, of a homogeneous deeply staining mass enclosing a few granules. For a time I regarded these masses as the food, and the strands stretching toward the lumen as direct evidence that substances were being taken up by the nucleus. In such cases as that shown in fig. 8, Plate XVI, which are numerous with various fixations, there seemed to be good reason for thinking that the substance of this mass is passing into or from a granular form, under the influence of the nucleus. Where, as in this cell, the nucleus lies far out in the cœlomic end, the mass seemed to be crowding it in that direction. A number of considerations have led me to abandon this view. In the first place, it was evident from Fischer's results that the food, when it enters the cell, ought to appear, according to Neumeister's scheme, in the form of granules, not as a heterogeneous mass; secondly, when the origin of the fine granules was traced to the nucleus, another constituent of the mass was accounted for; thirdly, when the alveolar structure was made out with certainty, the homogeneous constituent was seen to be interalveolar in position. In such cases as fig. 6, where the alveoles are particularly distinct, the strands are not to be distinguished from the fibres except by their extent. Not until the injection experiments, mentioned on p. 306, were tried was it possible to account for the position of the mass with reference to the nucleus. Fig. 8, Plate XVI, is from an intestine killed in the ordinary way by penetration from the cœlomic side; fig. 7, *A*, is from one which was injected with the killing fluid. Since we know that the interalveolar substance, to which all of the constituents of this mass belong, is free to move about in the cell, it is clear that the position of the mass is referable to the purely mechanical effects of penetration of the killing fluid. The nucleus acts as an obstruction to the streaming set up by the fluid; and in some cases a kind of eddy of the interalveolar substance is formed behind it. In other cases the mass projects beyond the nucleus; here it is possible that the interalveolar substances have been checked by reaching the limit of concentration allowed by the spaces, and have been "fixed" in that position. Only by some such supposition can I explain the remarkably straight edge presented by the mass on the side from which it came (figs. 6 and 8, Plate XVI). Still a further evidence that the mass is wholly artifact

is that it occurs with clearness only in the elongated cells, whose free ends are unprotected by a layer of muscle. We must conclude, therefore, that what in the first instance appeared to be so clear a case of cellular individuality in absorption, turns out to be only an artificial massing together of several of the cell constituents.

To return to the albumose, we have seen that it accumulates on the cœlomic side of the cell. It may be in contact with the basement membrane, and practically fill the whole cœlomic end. The next subject to consider is its fate. It has long been known for mammals that neither albumose nor peptone occurs in the circulation; in other words, the peptone is changed back to proteid *before* it reaches the blood stream. We might expect that such would be the case here also. The morphological support for this view is positive, so far as it goes. In extracting and fixing intestines some of the cœlomic fluid is invariably found precipitated on the outer surface; and in studying whole intestines, I have had occasion to precipitate large quantities of the cœlomic fluid on the slide: in no case have I seen albumose granules in the coagulum.

In several intestines the cœlomic end of the median cells was filled with a precipitate which might be described as mottled in appearance. There were no definite granules (Table I, Nos. 31 and 45), nor could the formation be described as a coagulum. Inasmuch as both these cases occur a considerable time after feeding, forty-two and one hundred and twenty hours respectively, we may suppose that the stage of the food represented is beyond albumose. Whether, however, the mottled precipitate is an imperfect granule formation, and this, instead of the small non-staining granules, represents the true peptone, or represents an intermediate stage in the inverse process toward albumen, I cannot say. In either case we would have good evidence that the food undergoes some change inside the cell. The intracellular ferment or ferments (for the zymogen granules may be of a complex nature) may be concerned in this change, in which case its association with albumose granules would be explained.

VI. Function of the Typhlosole.

We may properly discuss here the function of the infolded groove of cells which has been spoken of as typhlosole. The first mention of this structure which I have found in the literature is by Brand and Ratzeburg (28), where it is spoken of as a "furche" in which

the anterior part of the heart lies. Lereboullet (*loc. cit.*) described it as well as could be expected with the magnifying powers at his command, and recognized that it must be of some considerable significance in the function of the intestine. He says: "On ne saurait admettre que les rigoles soient destinées à augmenter l'ampleur de l'intestine; leur étroiteness et leur étendue limitée semblent contraires à cette opinion. Je ne crois pas qu'on puisse les regarder comme des organes particuliers de sécretion ou d'absorption" (*loc. cit.*, p. 91).

"Le seul usage probable de ces rigoles me parait être de recevoir une portion de la bile pour la conduire dans le milieu de l'intestine, afin que ce liquide se trouve répartir d'une manière plus uniforme dans toute l'étendue du ventricule chylifique. En effet quoique les rigoles ne se continuent pas directment avec les deux embouchures des utricules biliaires cependant on comprend que dans les mouvements de contraction de l'estomac, une partie de la bile puisse s'écouler par ces canaux. On remarquera d'ailleurs que le ventricule chylifique est presque toujours entièrement rempli d'aliments, en sorte que la bile peut éprouver de la difficulté à se parter jusqu' à l'extrémité de ce long boyau" (p. 92).

Ide says: "Nous n'avons recueille aucune donnée positive au sujet de la fonction de la bande dorsale. On peut dire sans hérésie que cette production augmente la surface d'absorption de l'intestine; nous avons constaté en effet, que les aliments au moins la partie la mieux trituree pénétrant dans les deux rainures qui longent la band" (*loc. cit.*, p. 189).

Conklin was the first, so far as I am aware, to apply the term *typhlosole* to this structure. By analogy with other invertebrate intestines, this name implies that the function of it is to increase the absorbing surface. Schönichen finally ascribes to the structure a possible secretory or excretory function, and supports this view by the fact that the dorsal blood-vessel breaks up into a plexus at the place where the "Rinnen-apparat" ends.

The observations with reference to the function of the typhlosole made in the course of this study are as follows: in the first place, the cells of the typhlosole are looser in structure than the other cells of the intestine (see p. 297). On this account they are very profoundly affected by the killing fluid. When artifacts do not occur in any other part of the intestine, it is a common thing to find the

typhlosole greatly ruptured (figs. 4, *B*, and Plate XVI, fig. 9). As will be seen from the same figures, the killing fluids act most powerfully on the inturned cells, just where the two streams of fluid, separated by the descending strip of muscle, are somewhat concentrated in their action. Often with sublimate-acetic and other very powerful reagents the upturned cells present horizontal "streams" of coagulated substance, which seems to indicate a movement of the cell contents toward the outside groove. I mention these artifacts because I was led by them at first to ascribe a *special* absorbing function to these cells.

Fig. 9, Plate XVI, represents albumose granules fixed in the course of passing through the cells of the typhlosole. We have then direct evidence that these cells share the functions of the others in the anterior end of the intestine, and confirmation of the view, expressed explicitly by Ide and implicitly by Conklin, that the infolding is for the purpose of increasing the absorbing surface. The quantity of both kinds of granules, however, is small as compared with that found in other cells. This may be due to the fact that in artificially fed animals the intestine was not gorged to the same degree of fullness which is common in the natural conditions. On the other hand, the fact that liquid food or secretion, or both, are often found in the grooves of the typhlosole in such intestines would indicate that these are not so highy specialized in these functions as the others, and would lend weight to the view expressed by Lereboullet that the furrows provide a means for the more ready passage of the secretion to the middle of the intestine, insuring thereby a more uniform mixture of the food and secretion. The direct evidence (exhibited in Table I) that the secretion is being poured into the intestine as long as forty-eight hours after feeding, and the facts, further: that under natural conditions the anterior end is often clogged up with bits of dry food; that in a freshly dissected animal whose intestine is full, the inner grooves are seen to stand up above the surface of the remainder of the intestinal wall, as if filled with something—plainly not solid food; that the character of the food, consisting as it does of substances often very difficult of penetration by the digestive fluids, requires the most thorough distribution of the secretion (which could scarcely be insured at the time of entering the intestine); and, finally, that the form of the typhlosole, narrow in front and widening behind, so that the grooves *may* permit the

escape of the fluid secretion all along the line, and *must* do so at the extreme posterior end (figs. 3, 4), is such as the distribution of the secretion would require—all these considerations seem to the writer to constitute a strong chain of evidence that Lereboullet's view is the correct one, namely, that it is the *primary* purpose of this structure to distribute the secretion. That it did not develop on the ventral side in direct connection with the opening of the glands, instead of on the dorsal side, is remarkable. Certainly, this would seem to be the more natural position for a structure concerned in the distribution of a secretion which enters on the ventral side; because it would be the more certain of finding its way into the channels if they occurred on this side. "Nevertheless," as Lereboullet says, "one can understand that in the movements of contraction of the stomach a part of the secretion (bile) may flow by these canals."

VII. Absorption of Carbohydrates.

This class of food substances is not at all favorable for a study of absorption by morphological methods. It is evident that none of the reagents ordinarily employed as fixatives act on carbohydrates in solution so as to render them visible to the microscope. A word on the digestion of starches, however, may not be without interest in this connection. Huet has found that the salivary glands (which Ide calls cutaneous glands) of *Ligia* produce diastase, and it is evidently to the secretion of this organ rather than to that of the hepatopancreas that he would ascribe the diastatic action in the intestine.[16] No effort has been made to confirm either Huet's or Ide's conception of the nature of the glands in question. It seems unlikely that a gland so small could have much to do with the action which we have now to describe.

Starved animals were fed on various kinds of starch,—cold-boiled potato, corn starch, dry bread, etc. The former proved to be especially palatable for some very large specimens of *Oniscus* which happened to be on hand at the time. They were allowed to eat freely of it for half an hour, in which time the intestine was completely filled. Twenty-four hours after they were killed, and the whole intestine subjected to Moore's test for sugar. Splendid

[16] Since he found the diastatic action of the "liver" secretion of *Ligia* only slight.

reactions for dextrose were obtained in a number of cases. Inasmuch as an effort was being made at the time to locate the dextrose in the cells, the other ordinary tests which require an alkali and heat were of necessity excluded. However, the reaction was controlled by trying intestines from animals known not to have eaten starch; and no indication of dextrose was obtained. Intestines were tested again fifty hours after feeding; and good indications were obtained.

Intestines like the above were tested also with iodine both twenty-four and fifty hours after feeding. In several cases the median arched cells gave a "port-wine" color at their free ends, indicative of either dextrine or glycogen, with the presumption in favor of glycogen.

VIII. Absorption of Fats.

In studying the absorption of fats the same feeding methods have been followed as for the absorption of proteids. Animals were isolated and starved from four to ten days, then fed with butter,

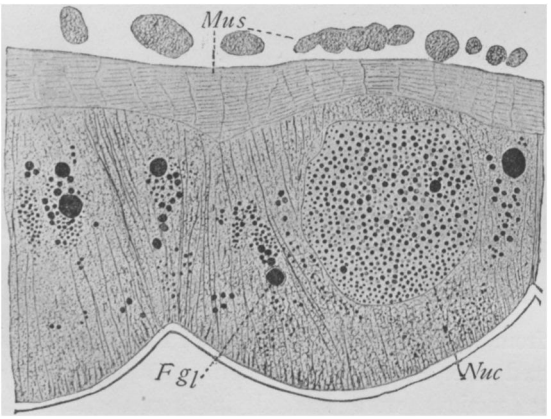

Fig. 15.—Section involving parts of two cells × 600 from anterior portion, "mid-gut" of *Porcellio scaber*, fed with butter, and fixed after 8 hrs. in 1 per cent. Platinic chloride 15 pts., 1 per cent. Osmic acid 4 pts. All black granules outside the nucleus are fat. *F.gl.*, fat globule encased by spherical film; *Mus.*, muscle, circular layer inside, longitudinal fibres outside; *Nuc.*, nucleus.

beef suet or olive oil (the latter mixed with boiled potato, to enable the animal to grasp the food with the mandibles), all of which gave

good results. As might be expected from the habits of land isopods, not all of the fat taken from a pure diet of this kind in the course of ten minutes' continuous feeding is absorbed. Pellets of waste containing a large proportion of fat were found forty-eight and seventy-five hours after feeding. But enough of the fat is absorbed, as may be seen from the figures, both to demonstrate the fat-digesting power of the hepatopancreas ferment and to follow the fat through the absorbing cells. Animals live indefinitely after eating the above-mentioned foods, and have been seen to eat the same repeatedly.

The digestion of pure fat, like that of pure proteids, is rather slow as compared with the action in mammals. At eight hours after feeding fatty globules may be seen in the epithelium of fresh intestines without the agency of stains. Fig. 15 shows an anterior intes-

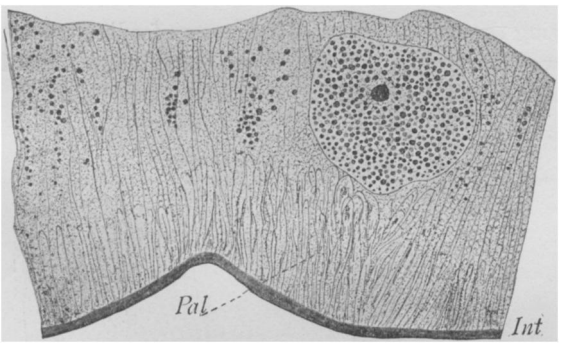

Fig. 16.—Section involving parts of two cells × 600 from intermediate portion, "mid-gut" of *Porcellio spinicornis*, fed with butter; intestine fixed after 12 hrs. as in fig. 15. All black granules outside the nucleus are fat. *Int.*, intima; *Pal.*, palisade.

tinal cell from an animal fed with butter for fifteen minutes, the intestine fixed after eight hours in Hermann's fluid without acetic acid. The sections were stained in iron-hæmatoxylin. The size of the globules varies greatly, the larger ones being doubtless due to the fusion of several small ones. It will be seen also that no large globules are found near the free luminal edge of the cell, but that in general there is an increase in size with the distance from this edge. This is typical of the appearance presented by all absorbing cells at an early stage in the absorption. Later, as is shown in fig. 16 (12 hrs.), there is not so much difference in size.

From twelve hours after feeding onward, just as in the proteid absorption, the food is usually aggregated mainly on the cœlomic side of the cell (figs. 17, 16 hrs.; 19, 115 hrs.), although as in figs. 18 (50 hrs.) and 10, Plate XVI, (24 hrs.) it *may* be still widely

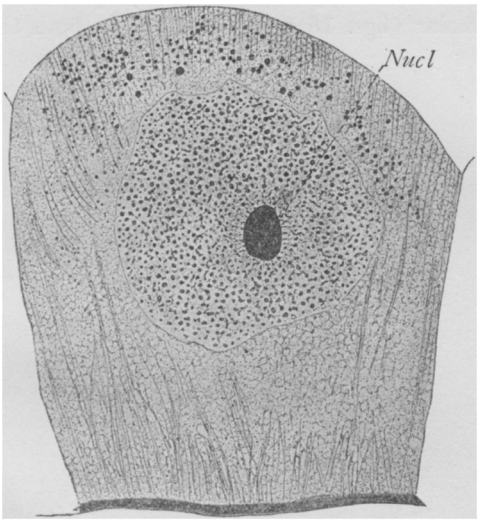

Fig. 17.—Section of cell × 600 from median portion, "mid-gut" of *Porcellio scaber*, fed with butter; intestine fixed after 16 hrs. as in fig. 15. All black granules outside the nucleus are fat. *Nucl.*, nucleolus: the chromatin is precipitated in short radiating strands immediately about its periphery.

scattered through the cell at a much later period. The difference here is due in part to the different kinds of fat used—butter in figs. 15, 16 and 17, beef suet in 19 and olive oil in 10, Plate XVI—and in part to the difference in form of the cells in anterior (15), intermediate (16) and median (18) portions of the "mid-gut."[17]

Just as in the absorption of proteids, the passage of food through the median cell is facilitated by the relatively easy exit from the cell into the cœlome, while from the anterior cells the passage is hindered by the investing muscle layer. Consequently at any given time after the digested product has reached all the cells, the median

[17] The position of the fat in the cell is not affected by the direction of penetration of the killing fluid, as may be seen from the fact that fig. 18 is from an intestine injected from the posterior end with Hermann's fluid. There has been no movement of the fat on account of penetration from within.

cells show a more advanced stage of absorption. Here also the amount of food found at any given time in any particular cell is dependent on the accidents of position, of form and of digestive action in the lumen.

The relation of the fat globules to the cytoplasmic structure is again interalveolar (figs. 18 and 19, *A*). In both these figures

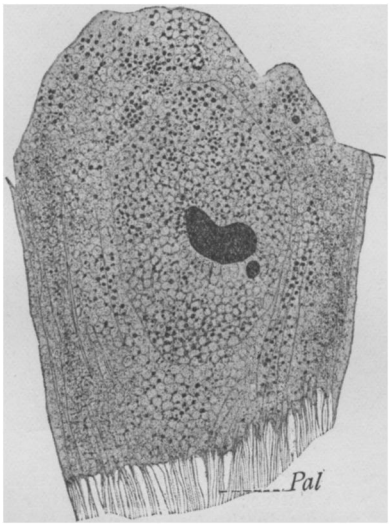

Fig. 18.—Section of cell × 600 from median portion, "mid-gut" of *Porcellio scaber*, fed with butter; intestine fixed after 50 hrs. in Hermann's fluid by injection from the posterior end. Nucleus is imperfectly fixed, and cytoplasm is slightly diagrammatic. Fat globules seldom inside alveoles. *Pal.*, palisade; intima torn off.

fat globules appear to have penetrated the nucleus. This appearance is due in the former to imperfect fixation, the chromatin not being all precipitated (see section on structure of the nucleus); in the latter the globules are in reality above, *i. e.*, at a higher focus than the nucleus. No fat granules are ever found in the nucleus.

This last statement may fairly raise the question of the identification of fat globules. In sections from intestines treated with osmic acid and stained in iron-hæmatoxylin (fig. 18) how, it may be asked, are blackened fat globules to be distinguished from stained chromatin granules? Or, more broadly considered, can the fat be distinguished at all by color reactions? This question is important

because upon its answer depends, largely, the physiological interpretation of the facts here presented.

In the first place, it should be said that the choice of iron-hæmatoxylin stain for the study of fat absorption was for the sake of very decided advantages from the morphological standpoint. The demonstration of the alveolar structure of the cytoplasm, for example, is much more satisfactory with this stain than with any other I have used. Fat globules may be quite clearly distinguished from other granules, black after osmic acid and iron-hæmatoxylin, by merely destaining to a considerable degree with the 1 per cent. iron-alum solution. Blackened deeply by osmic acid, they retain their color after other granules blackened only by the stain

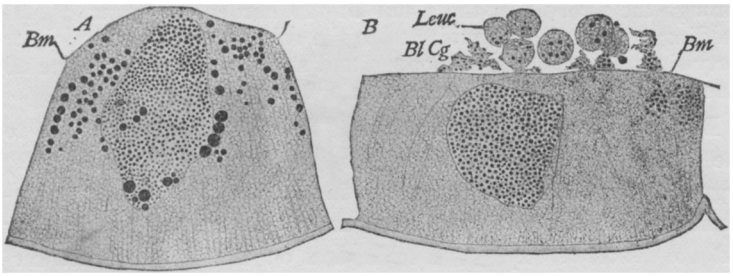

Fig. 19.—Sections of two cells × 600 from median portion, "mid-gut" of *Porcellio scaber*, fed with beef suet; intestine fixed after 115 hrs. in Flemming's fluid. *Bl.cg.*, blood coagulum; *Bm.*, basement membrane; *Leuc.*, leucocytes containing fat. All black globules outside the nucleus are fat.

lose the color. This is the case in fig. 18; the black color in the fat granules is due solely to the osmic reaction.

Secondly, with acid fuchsine stain it is an easy matter to distinguish granules from fat globules. In fig. 10, Plate XVI, they are shown retaining the osmic color, while all the other cell parts are red. The nuclei which are not shown in these two cells were also red. Figs. 17 and 19 likewise are from sections stained in acid fuchsine; the fat globules were all black, the nuclei red. It has been seen (p. 315) that albumose granules take the acid fuchsine in aniline water even after an osmic fixation. It is consequently easy also to distinguish the fat globules from albumose granules. The food was pure fat or fat and carbohydrates mainly, and sections treated in the same manner as those in which albumose was

demonstrated by this stain (namely, two to three minutes in a 7–15 per cent. solution in aniline water) always exhibit the blackened granules only. In fig. 10, Plate XVI, some small red granules are seen near the free edge of the cell; these may be due to the small amount of proteid in the boiled potato with which the olive oil was mixed, or may represent small globules composed in part only of fat.

Thirdly, the final test of fats is always the action of fat solvent —ether, xylol, benzole, etc. After most fixations, as is well known, fat is easily dissolved out by prolonged immersion in xylol or ether.

Thus the intestine from an animal known to have eaten only fat after a starvation of one week was fixed after twenty-one hours, first in 1 per cent. platinic chloride, washed in water and carried through the alcohols in the usual way to harden. Instead of imbedding at once, it was subjected to the solvent action of xylol for forty-eight hours. On sectioning and staining in acid-fuchsine no blackened globules were found in any of the cells.

But after osmic acid fixations the authorities are not agreed as to the solubility of fats in the ordinary solvents. Heidenhain (29) argued that certain black granules in the absorbing cells of mammalian villi and in the lymph corpuscles were not fat because they did not dissolve in xylol and ether, and on this fact he based certain far-reaching conclusions. Altmann (30), however, and Moore in Schafer's *Text-book of Physiology* (Vol. I, p. 458, note 5), state that prolonged treatment with osmic acid tends to render fat insoluble. This I can confirm from the following experiment concerning fat globules in cells:

Intestines from animals which ate butter for eight minutes after a starvation of one week, fixed in Hermann's fluid without acetic acid after sixteen hours and treated with xylol, exhibit abundant blackened granules. In one case (fig. 18) the intestine was fixed in Hermann's fluid, and after the sections were mounted one of the slides of the series was sketched with the camera while in oil of cedar in preparation for balsam. The position of every globule in the cell was noted. It was then placed in ether over night (fifteen hours) and was again examined in oil of cedar. No change in the globules had taken place. The slide was then placed in benzole for four or five hours and again carefully examined; no globules had dissolved, although the color had faded noticeably. There is no possibility of confusion with albumose granules in this case, as the animal was carefully isolated for nine days before feeding and was then allowed to eat only butter for ten minutes. It was killed and

the intestine was fixed after fifty hours, when many globules are still to be seen in other slides of the series.

The first experiment mentioned above furnishes additional proof that the globules found in the cells are fat. Since there can be no question about the identity of the globules in the second experiment, it shows that osmium-fixed fat globules are practically insoluble in ordinary fat solvents. This is not true of fat outside the cells—that is, while still in the lumen.

Sections of intestine of an animal fed for twenty minutes on butter and fixed after seventy-five hours in Flemming's fluid without acetic acid, were mounted and stained in acid-fuchsine in the usual way. Instead of mounting in thick balsam, a large amount of oil and a little balsam were placed on the section. The next day the fat, densely blackened and enclosed by the epithelial wall, was seen to be diffusing out, and in a week it was entirely dissolved by the excess of cedar oil. The cells of the epithelium, however, still contained abundant globules blackened by the osmic acid.

That the globules last mentioned did not dissolve may be explained by supposing the fat inside the cell to be mixed with some coagulable substance.

Butter spread on a cover-glass and treated with Hermann's fluid for fifteen hours, then with ether (after dehydration), leaves a coagulated residue which retains its black color for more than two days in the solvent. Again, in teasing out in Hermann's fluid an intestine which had been filled with olive oil, it was observed that the oil, mixed with the secretion of the hepatopancreas, on escaping in the form of globules, took a brownish color at the periphery and the densely black color within. The brown color may have been due to fatty acid or to a film of the coagulated secretion.

Finally, as Altmann has observed, decomposition products or other diluting substances are probably responsible for the different degrees of intensity with which the osmium-blackening occurs or remains after treatment with solution agents (*loc. cit.*, p. 98). It is scarcely possible to suppose that the globules of fat inside the cell are wholly unmixed with the albuminous fluid contents of the cell, or with the immediate products of digestion.

The whole evidence for fat in the cells may be summarized as follows: (*a*) Oily globules are seen in the cells of fresh intestines from animals fed with fat; (*b*) these globules are dissolved by xylol after fixation in $PtCl_4$ (and certain other reagents—$HgCl_2$,

chromic acid, etc.); (*c*) they stain densely black after all osmic fixations; (*d*) they retain this color after staining with acid-fuchsine in aniline water, and are thus distinguished from albumose granules; (*e*) they retain the black color after iron-hæmatoxylin and destaining with iron-alum, and are thus distinguished from chromatin granules, so that the granules inside the nucleus, which physically very much resemble the blackened granules in such preparations, are never oily in nature—in other words, the fat does not penetrate into the nucleus in appreciable quantities; (*f*) the insolubility of these granules in xylol and ether cannot be taken as an indication that they are not composed in part at least of fat, for, as Altmann observes, the solubility depends on the purity of the fat. Finally, the insolubility of globules in such cases may be due to admixture with some albuminous fluid which precipitates in the formation of the globule and constitutes its insoluble portion.

Granting now that the globules found in the absorbing cells after feeding with pure fats are really fatty in nature, it may be inquired how they came there, whether they are absorbed as such or whether they were synthesized from chemical products of digestion. In short, for which theory of fat absorption—the emulsion or the solution theory—do the facts speak? Without going into a history of this controverted question here, it may be pointed out, by way of introduction to the interpretation of what follows, that the morphological evidence is scarcely crucial evidence in point. For, on the one hand, it is claimed by the adherents of the solution theory that if fat globules are not demonstrable in the luminal border of the cell, but only after a portion of the cell has been traversed, then fat did not enter the cell *as such*, but has been synthesized somewhere in its course since entering. The emulsionists, on the other hand, say it is just as reasonable to suppose that the emulsion particles are so small that at the time they enter the cell they cannot be seen with the highest powers of the microscope, and become visible only when a number of them have fused together, as they plainly do.

The isopod, because of its small size, is not favorable for a chemical study of digestion. Consequently I have not attempted to supplement the morphological study by that means. Nevertheless, it seems worth while pointing out that the appearance here presented is all that is required by the solutionists for morphologica

support to their view; while it is difficult to reconcile it with the emulsion view. Pflüger (31) has used Heidenhain's figures to show that fat does not appear in the striated border of mammalian epithelial cells, and he declares, quoting Funke (32) and Will (33), that it has never been so demonstrated except by Kölliker (34), who, as Pflüger says, wanted to see fat particles to substantiate his theory that the striæ are pores. My own observations on this point in the isopods were begun with the purpose, primarily, of demonstrating the pores in the chitinous lining (see p. 310). It was hoped that if fat is absorbed in the form of emulsion, it would blacken densely in the intima after osmic acid. Consequently particular attention has been given to this point. About twenty-five intestines from animals in all stages of digestion, from four hours to 145 hours after feeding, have been sectioned and each one examined carefully with this point in view. In no case have I seen a single cell whose intima contained demonstrable fat globules. (Note that in fig. 10, Plate XVI, the intima is stained with acid-fuchsine.) It has been previously mentioned that the globules on the luminal side of the cell are always small in size, while they increase gradually farther up the cell. This is manifestly what is required by the solution theory, as has been recognized by Funke, Will, Altmann, Krehl (35), Pflüger and others. If fat enters the cell in the form of fatty acid and glycerine, or soap and glycerine, and these are then synthesized into neutral fats under the influence of the cell, the neutral fat would naturally appear first in small globules at the luminal edge, and these would increase in size or in number, or both, the more the products of digestion were brought under the synthesizing action.

It cannot be denied, as urged by Heidenhain and others, that if fat did enter the cell in the form of a fine emulsion, and were then to fuse into larger globules, the same appearance might be presented; but there is no assignable reason then why larger globules are not formed in the membrane (intima of isopods) or on the luminal side of the cell. In line with Will's results, it may be remarked further that beef suet does not melt at the temperature of the isopod body (about 25° C.), and cannot therefore be emulsified. Hence the globules in fig. 19 must result from synthesis.

On the question of what effects the reverse action—the synthesis of the products of digestion into neutral fats—I can do little more than conjecture with others. Until recently no attempt has been

made to seek out this agency further than to ascribe it to the epithelial cells (see Moore's review of the subject in Schäfer's *Text-book of Physiology*, Vol. I, p. 452).

Recently, however, Kastle and Loevenhart (36) have shown that the ferment lipase extracted from the pancreas of the hog has the power of bringing about the synthesis of ethel-butyrate from ethyl-alcohol and butyric acid—a reaction evidently equivalent to the synthesis of palmatin or stearin from glycerine and palmitic acid or stearic acid.

In considering the fate of albumose in digestion of proteids (see p. 317), I have ventured to ascribe a ferment nature to certain granules derived from the nucleus. It may be conjectured that some of these granules represent a ferment which has a fat-synthesizing action.

Prof. Moore, in Schäfer's *Text-book* (Vol. I, p. 457), says it is agreed by all authors that "fat passes from the epithelium . . . . in the form of an emulsion," and my acquaintance with the literature confirms this statement. I have found nowhere any intimation that the fat does not pass out of the cells as such. Even Levin (37), who maintains that the water-soluble products of fat digestion in the dog are taken up by the lymph cells only, and are by them conveyed directly to the lacteals, while the epithelial cells are stimulated by the bile and pancreatic juice to take up the fat itself, leaves the inference that the fat reaches the lacteal in the form of an emulsion. The evidence for this very general opinion is plainly that fat is always found in the form of globules on the way from cell to lacteal, either in the parenchyma cells of the villus or in the lymph cells. Heidenhain, Schäfer (38), Levin and many others have figured fat in transit in this form, and the very name of the vessel which receives the fat is witness to the multitudinous observations that the fat reaches it in the form of tiny globules or an emulsion.

Does it, however, follow that because the fat reaches the lacteal as an emulsion or is found on its way thereto in this form, that it therefore leaves the cell as such? Does not the very physiological reason for the splitting of fats into fatty acids and glycerine preclude the possibility of its passage through the basement membrane, unless we assume with Brücke (39) that there are discrete channels for the passage of the food to the lacteal? If it is true, as Moore intimates, that fat leaves the cell as such, then it ought to be preserved

in the basement membrane by the osmic reagents. I have examined carefully all my preparations with the hope of finding some such evidence, but have not found a single cell in an intestine known to have contained fat which presented the expected appearance. In one or two cases where the animal was fed with raw beef, in which there may have been a slight trace of fat, the basement membrane was found filled with small granules which blackened densely with osmic fixations; but as judged by the number of globules in the cells after a full meal of fat, there were entirely too many of these for the small quantity of fat which it is possible to suppose may have been contained in the beef, since particular care was being exercised at the time to feed with lean meat free from fat. Moreover, the cells presented coincidentally with these globules fragmented nuclei,—probably a sign of degeneration (see p. 294).

It is significant that while I was confidently expecting to find fat globules in the basement membrane, none of the drawings made at that time and reproduced here shows blackened globules even in contact with the membrane, while some of them (fig. 18) show a gradation in size downward from the region of the nucleus to the basement membrane. At this time—sixteen hours after feeding—fat was passing through the membrane, probably in small quantity only, but in fig. 19, *A* and *B*, both from the same intestine, 115 hours after feeding, it must have been passing in considerable quantity. In *B* of the last figure, fat globules are seen lying against the membrane on the outside of the cell; but here, it must be said, the blood was precipitated on the intestine by removing the dorsum and fixing the intestine *in situ*. Only in such cases have I found fat globules immediately against the membrane in the coagulum outside the cell. This coagulum is often found in this position even on intestines fixed after removal from the body. The morphological evidence, therefore, is against the passage of fat through the membrane *as fat*. The conclusion must be that it is again split up in the cell and resynthesized in the cœlomic fluid.[18]

The same figure shows several leucocytes containing fat globules. Their position along the membrane cannot, of course, be taken to indicate a special agency in removing the fat from the cell, for

[18] Shortly after reaching this conclusion I received Loevenhart's (40) paper, setting forth the same view from very different considerations.

they are plainly subject to the physical action of the fixing fluid. The fat globules are inside the cells, however, and this must be accepted as evidence of their ability either to appropriate fat in their own metabolism, or to transport it in the metabolism of the body. I have seen nothing which would enable one to decide whether this action is anabolic or katabolic.

IX. Secretion in the Hepatopancreas.

Weber (41), who first accurately described the hepatopancreas, recognized in its walls four distinct layers: The *serous membrane* outermost, the *muscular* between this and the *basement membrane*, and finally the *epithelial* layer. While it is the last named only with

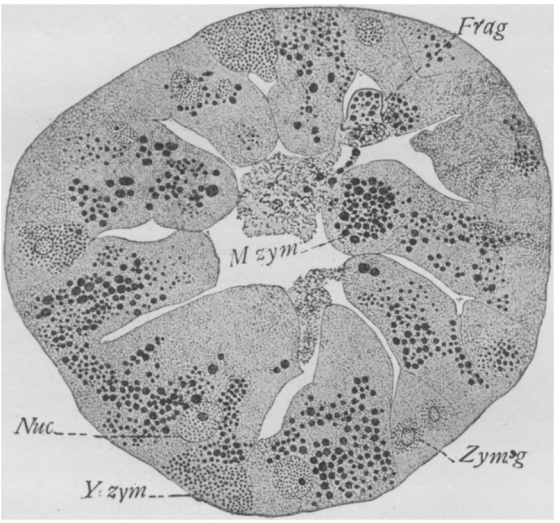

Fig. 20.—Section of a single tube of the hepatopancreas of *Porcellio scaber* × 120, showing discharge of secretion by fragmentation and by rupture of the cell membrane. *Frag.*, fragments of cell passing into the lumen; *M zym.*, mature zymogen passing into the lumen by rupture of the cell membrane; *Y.zym.*, zymogen in a young secreting cell; *Zym'g.*, zymogenesis in a young cell; *Nuc.*, nucleus of a mature cell.

which we are specially concerned here, it may be mentioned in passing that Weber's explanation of the form of the tubes by the arrangement of the muscles is important as bearing on the discharge of the secretion into the intestine (fig. 1). He shows in his figure that the spiral twist which the tube appears to have

undergone is due to the collection of muscle fibres into a more or less distinct band which takes a spiral course. Manifestly the peristaltic wave, beginning at the distal end and proceeding with greatest vigor along this band, will be more effective for producing a uniform and constant motion of the fluid contents than would the successive contractions of many separate bands.

The epithelium in the average physiological condition is composed, morphologically speaking, of two kinds of cells, namely, tall conical cells which project well into the lumen (often, indeed,

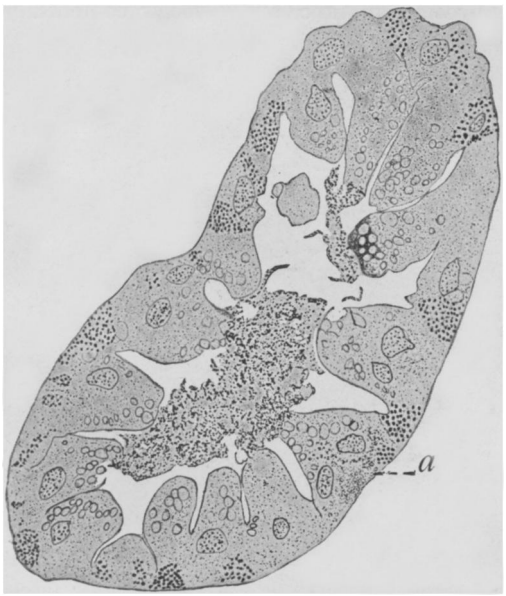

Fig. 21.—Section of single tube of the hepatopancreas of *Porcellio scaber* × 120, showing discharge of secretion by fragmentation of the cells. The cell *a* is represented with higher magnification in fig. 22.

almost meeting each other so as to obliterate the lumen) and, between these, lower cells which project less—often not at all (fig. 20). At both extreme ends of the tube the two forms of cells merge into one another and thereby constitute a uniform epithelium which, at the distal end, terminates in a proliferating mass of indifferent cells, and, at the proximal, passes over into the epithelium lining the grinding stomach. The cells of the hepatopancreas reveal a ground structure in some points like that of the

intestinal cells. Examined fresh, large oily-looking globules are always to be found in the projecting cells, which, after most fixations, are represented by mere vacuoles. These increase in size from the base toward the apex, and often quite completely occupy the apical end, displacing the ground structure itself (fig. 22). Between the vacuoles, in well fixed cells, are to be seen small alveoles which, if the vacuoles be large and close together, are more or less distorted by the pressure. Between the alveoles and covering them, often so densely as to obscure them, are tiny granules representing interalveolar substance and alveolar contents. After some

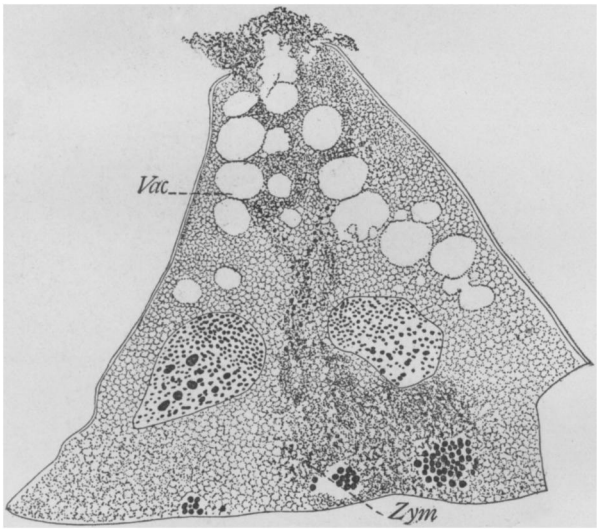

Fig. 22.—Cell *a* of fig 21 × 600. Escape of the secretion by rupture of the membrane at the free end of the cell. *Vac.*, vacuoles from which the globules of zymogen have been dissolved by the fixing fluid and other reagents; *Zym.*, zymogen granules disintegrating.

fixations, notably those containing sublimate, the cells appear to have a coarse fibrillar structure; but as this is not to be seen either in the fresh cell or after Hermann's, Flemming's, or Hermann's without acetic, it has been regarded here as an artifact similar to that produced in the intestinal cells.[19]

[19] Frenzel (46) sees a fibrillar structure in the "liver" cell of the marine isopods, relying on picro-sulphuric fixation. He does not, however, figure this for *Oniscus murarius*, on which he used osmic acid.

Each secreting cell has one or two, rarely three, nuclei. When there are two, which is by far the most common number, they are most often abreast of each other, at the same height from the base and at equal distances from the lateral walls (fig. 23). The form

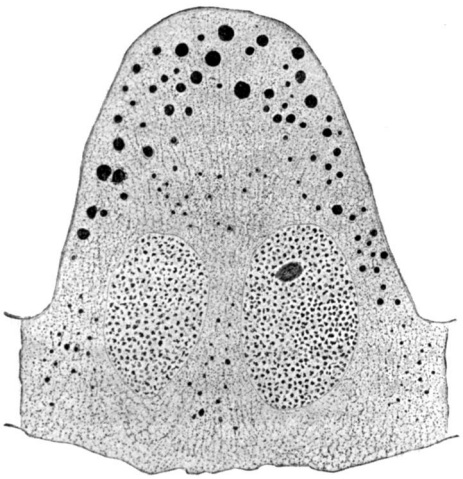

Fig. 23.—Section of a mature cell, × 600, of the hepatopancreas of *Oniscus asellus*, only moderately filled with zymogen. The gradation in size of the granules from base to apex of the cell is well seen here. Fixation, Flemming's fluid. Observe a space about each of the larger globules. Compare fig. 15.

of the nuclei in the fresh condition is ellipsoidal or spherical. After several fixations it may present processes of various size and extent, but these again are to be ascribed to the unilateral penetration of the fluids (p. 305).[20]

[20] Prenant (42) has mentioned such processes toward the base of the cell as occurring after Flemming's fixation, and has interpreted them as analogous to those which were described by Conklin in the intestinal cells; also to those described by Korschelt for the nuclei of silk glands of the Lepidoptera and of the egg cells of *Dytiscus*. The fact that the processes are turned toward the source of nourishment and opposite the direction of penetration lends some probability to Prenant's view, whereas, in line with the results obtained by injection into the lumen of the intestine, one would expect the processes in this case to extend toward the lumen if caused artificially. In the absence of positive evidence from the experiment of injecting into the lumen of the hepatopancreas, which is very difficult on account of the small size of the tubes, it might be urged further in explanation of Prenant's observation, first, that Flemming's fluid is known to cause processes in the nuclei of the intestinal cells; secondly, that occasionally in these cells processes are

The constituent which gives character to the cells under consideration is the zymogen (figs. 20–23). In the fresh condition it is always seen as a dense mass of spherical yellowish granules surrounding the nuclei of the smaller cells. They are but rarely seen in the tall cells, the most obvious constituent of these being, as we have observed above, the oily-looking globules. On this account Weber called the smaller the "ferment cells," as opposed to the larger or "liver cells." Rosenstadt (44) for *Asellus*, Giard and Bonnier (45) for the parasitic Isopods and Frenzel (46) for the marine forms, as well as for *Oniscus murarius*, do not admit this distinction made by Weber. Claus says they are only extremes of the same kinds of cells, in no way to be distinguished. Frenzel regards the smaller as young cells and the larger merely as a later or older phase. He concludes, therefore, that the isopods are to be classed with the Phronimidæ, in that they produce both ferment and fat in the same cell, as over against the decapods, the Gammaridæ and Caprellidæ, which produced these in separate cells. Ide is inclined to adopt the view of Weber. My results confirm Frenzel, as will appear in the following discussion.

The behavior of the zymogen granules with reference to reagents is as follows. As was observed by Huet, they are speedily dissolved out by both water and alcohol. Reference to Table I, where are brought together data from a long series of different physiological conditions, shows that they are not preserved by alcohol (Nos. 24, 36, 38), only partially by picro-acetic (29, 43), and sometimes not by sublimate (28, 31), nor by sublimate-acetic (3, 9, 11, 13, 22, 41, 42, 44), nor formo-alcohol (32). They are always preserved by Flemming's, Hermann's and Hermann's without aectic (also osmic-acetic and osmium-bichromate, Altmann's). It will be seen also from the table that they are sometimes preserved in the small cells and not in the large ones (Nos. 11, 13, etc.), sometimes again in both kinds of cells, where they appear precisely alike (Nos. 15, 18, 26, etc.). With the osmic fixations, particularly the platinic-chloride-osmic acid mixture, they behave a little differently in the two kinds of cells. The densely aggregated granules about the nuclei of the small cells come through the stains wholly un-

seen extending opposite to the direction of penetration, while they are also occasionally seen in the cells of the hepatopancreas, extending toward the lumen after fixation; thirdly, as was remarked in the beginning, nuclei in the fresh condition are regularly curvilinear.

affected, exhibiting a brown color, plainly the characteristic osmic color. Those in the base of the large cells, on the other hand, take the stains. Thus in Nos. 21, 25, etc., stained in iron-hæmatoxylin, they appear brown and black respectively, while in No. 35, stained in acid-fuchsine, brown and red. After picro-formalin also (No. 16) those in the large cells stain red in Biondi-Heidenhain, while those in the small cells remain unstained.

In Nos. 1 and 2 there is a complete transition from one color to the other, some retaining no stain, others retaining only a light shade or only a peripheral ring of black, still others holding it densely. Moreover, the transition does not stop at granules of the same size; among those in the large cells which hold the stain most tenaciously there is a perfect gradation from small granules to large globular masses (fig. 20). The latter correspond to the contents of the vacuoles mentioned above (fig. 22). We have evidence, therefore, that the two kinds of cells are, as Frenzel holds, but the young and mature phases of the same kind. Our evidence goes a step farther. Frenzel found both zymogen granules and fat globules in the same cell, and from this concluded that all the cells produce both, the ferment during the early life and fat later. Now we have been able to trace a complete transition from the zymogen granules to the large globules, merely by securing a good fixation for all the cell constituents. The indication is, therefore, that we have to do not with two distinct products, but with different stages in the formation of a single product.

Before going farther with the present discussion it will be necessary to present the changes which the cells undergo in secretion. Fig. 21 represents a cross-section of No. 10 (Table I) preserved twelve hours after feeding—this following a fast of eleven days. In this case the large globules are not preserved, only their vacuoles being seen. At the bottom of the cells are masses of zymogen granules, some of which are becoming less distinct in outline, others are represented merely by a dense mass of small granules. Fig. 20 (No. 20), sixteen hours, after a fast of twenty-one days, shows the large globules preserved. In the former case, as well as in the latter, the ends of some cells have broken down and are undergoing a process of disintegration. Sometimes the whole end of the cell is involved in this destruction, or the end may break up into large or small fragments, or finally break off as a whole, and the larger or smaller pieces are then found in the lumen as far down as the canal

which leads to the intestine, where they may lodge for a time and temporarily block the passage. In other cases still the globules only seem to be set free; while *the small zymogen granules seldom come near the discharging end.* The nuclei are not lost, nor is any part of the cytoplasm below them.[21]

After complete discharge the cells diminish rapidly in height until they are quite flat. Even at this size they are easily to be distinguished from young cells, which may as yet be no taller than they, by the absence of small zymogen granules about the nucleus, by the shape of the globules or globular spaces, and by a thickening of the free edge (when this is not lost). The last two effects are caused by a retraction, as if the cells were elastic. When discharged the spaces occupied by many of the globules are obliterated, and a thickening or moving together at the free edge occurs, forming a layer which always stains densely (fig. 21) (this is the thickening just mentioned). Those globules which are not discharged suffer a change of form by compression, so that they are always laterally elongated. I have not so far been able to follow the fate of the discharging cells further than this, or to obtain further evidence that they are completely destroyed and replaced by new cells.

It will be observed from the table that discharging cells may occur at any interval after a single meal up to 124 hours (No. 44), although there is a marked decrease in the number after forty-eight hours, and in some cases hardly any are to be found at ninety hours (Nos. 40, 41, 42).

In a single case (41) some of the young cells seem to be discharging a fluid substance; but as the fixation is one which does not always preserve the zymogen, and it has not been confirmed by any perfectly trustworthy fixation, no account has been taken of it here.

By whatever process the discharge takes place—fragmentation, dissolution, or mere evacuation—in every case there issues the fluid whose precipitate gives the characteristic coagulum. Fig. 20 shows some of the globules maintaining their identity for a time, but sooner or later dissolving. They are not the only source of the fluid, for, as in fig. 22, which contains a very abundant interalveolar substance, this as well as the alveoles of the cells contribute to its formation. As we have already observed, small zymogen granules,

[21] In one or two cases of excessive feeding, where the cells become enormously gorged with a substance which stains blue in Biondi-Heidenhain, after Zenker and sublimate, the nuclei may be displaced and lost in broken-off fragments (Nos. 14 and 46, table).

which are readily distinguished by color, do not contribute directly. Some of these appear to dissolve without being confined to a globule (as in the bottom of fig. 15), to be added to the interalveolar substance which precipitates in the form of very fine granules. Close comparison of this with the precipitate from the globules, as well as close scrutiny of the precipitate found in the lumen, fails to reveal any difference corresponding to granules and globules.

It would be unwise to attempt, from the facts thus far reached, to show that there is no chemical difference between the various cell constituents which ultimately share in the formation of the secretion, or still more that no corresponding differences are retained throughout the existence of the secretion. There is every reason for thinking that the secretion is far from simple in nature. So far as they bear on the occurrence of fat, however, the facts just rehearsed seem to speak very positively. Both Weber and Frenzel base their assertion that the globules contain fat on the osmic reaction and on the action of fat solvents (ether). Nothing is here urged against the facts alleged by these authors. What seems fatal against concluding therefrom that the substance in question is pure fat is that, as we have seen, it is never represented in the coagulum of the secretion by globular spaces such as fat ought to leave, and such as it always does leave when dissolved after fixation. The globular masses which *sometimes* come out of the cell as such, and do exist in the fresh secretion as oily-looking drops, invariably, so far as my observation goes, precipitate as a dense coagulum, not to be distinguished from the remainder of the secretion. What then is the nature of the globules? Aside from their oily appearance, nothing occurs in the literature reviewed which throws much light on their nature as they are to be seen in the fresh condition. Sufficient prominence has not been given either to the fact that in this condition they are to be seen in all sizes. They are usually much clearer looking than the zymogen granules, but even in this respect the limits are not sharp. Frenzel mentions the fact that the oil globules in *Ione, Gyge, Idotea hectica* and *Sphæroma* have almost always a definite color, "namely, in the first three greenish-yellow (*wie das Secret*), in the least one a brownish-yellow."

The conclusion which appears very evident from the foregoing is this: zymogen granules are merely young stages of the large globules or, conversely stated, the globules are but a matured state of the zymogen granules. The difference as to form, color, behavior

toward fixing fluids and staining capacity is due to a difference in purity. The granules, densely packed about the nuclei of the young cells, are in a nascent state, consisting of a pure ferment-forming substance. In the larger cells—that is, as the young cells mature—the zymogen takes up fatty substance from the cytoplasm, and this dilutes the ferment-forming body, at the same time rendering it more soluble in certain reagents and improving its capacity for certain stains.

a.—Relation of Secretion to Feeding.

The column of Table I headed "Secretion in lumen" exhibits the relation of the quantity of secretion in the lumen and its passage into the intestine to the stages of absorption of proteids (raw meat) exhibited in the last columns. Owing to the method of removing from the animal the intestine and hepatopancreas, by which their connection is often broken, the later datum was not in all cases to be had. It will be seen, however, that beginning with fresh condition—that is, animals taken quite at random from the natural habitat—and passing through the starved condition (No. 3) up to 124 hours after feeding, and to an animal kept a whole week on raw meat, there is almost always an "abundance" of the secretion in the lumen. In a few cases, as No. 3 (starved) and 42 (ninety-eight hours after feeding), the quantity in the proximal end of the tube is small. There is no very marked decline, although it might well be expected in the course of another day or two of fasting.

The chemical reaction of the secretion, as determined by teasing the "liver" on litmus paper, is slightly alkaline for animals taken from the natural state. The staining affinity of the secretion seems to vary. Thus in 13 and 22, both of *Oniscus*, starved twenty-one days and killed sixteen and twenty hours respectively after eating in the same fluid and treated precisely alike (stained in Biondi-Heidenhain), in the one case the secretion takes the methyl green, in the other the plasma ssain and comes out red. A number of such intestines have been seen, and both colors have been seen in the same intestine. If the staining is a chemical reaction, and there seems to be no doubt of it in this case, we must conclude that the reaction of the secretion must vary from acid to alkaline (as determined by Biondi-Heidenhain stain as an indicator). Not enough cases have been noted to establish any cycle of such change, or to relate it in any way with the quantity or kind of food. It is inter-

esting, in this connection, to note that Krukenburg (26) has found a similar difference for *Carcinas mœnas.*

Huet has shown that the secretion of the hepatopancreas acts on the proteids (muscular fibre and albuminoids generally, white of egg, coagulated protoplasm of vegetable cells, etc.) in *Porcellio scaber* and *lævis;* on starch very slightly in the case of *Ligia;* while on fats he obtained only negative results (slightly emulsifies oil of olives). He concludes that the secretion is " not a true bile."

It has been seen in the section on the absorption of proteid that albumose appears in the intestinal cells in eight hours (in one case it was detected in four hours) after feeding. No attempt has been made to establish ultra-minimum time for the appearance of dextrose in the intestine after feeding with starch. The least time noted was twenty-four hours. There is good evidence that the " liver " secretion accomplishes the hydrolisis of fat. We may conclude that the secretion of the hepatopancreas contains ferments which act on all classes of foods.

X. Summary and Conclusions.

1. The apparatus concerned in the absorption of foods and the production of secretion in the land isopods consists essentially of simple tubes, the intestine and hepatopancreas, bathed and separated by the blood.

2. That part of the intestinal wall concerned in absorption is a single-layered epithelium composed of very large cells. That part of the hepatopancreas concerned directly in the formation of secretion is a single-layered epithelium, composed of smaller, young cells and larger, maturing or matured cells.

3. The intestine in the freshly hatched individual, a simple tube, grows by amitosis, especially at the time of moulting, as well as by enlargement of the cells. In the mid-dorsal line of the anterior portion a folding of the wall occurs later, giving rise to a structure which we have called, after Conklin, the typhlosole. The typhlosole arises in *Porcellio spinicornis* by a primary evagination of the median six longitudinal rows of cells; there is then a secondary invagination of the median two rows, which project into the lumen and extend laterally by their free margin so as to cover the lateral inner grooves formed at the sides, thereby cutting off, except at the posterior end of the typhlosole, two channels, more or less completely separated from the remainder of the lumen.

4. The intestinal epithelium is a syncytium, the cytoplasm being continuous from one cell to another. Intercellular fibres, together with furrows in the basement membrane and intima, serve to mark off the cells distinctly.

5. The cytoplasm of the intestinal cells is alveolar in structure. Between the alveoles is a homogeneous substance which precipitates as a finely granular coagulum. Intracellular fibres run between the alveoles; at the luminal side of the cell they are parallel and are greatly thickened so as to form a palisade from the intervals in which the cytoplasm may be excluded.

6. The nucleus of the intestinal epithelial cells is normally spherical in all physiological conditions; it is alveolar in structure in the fresh condition, and contains in "perfectly" fixed material numerous large granules of chromatin.

7. In the moult of the chitinous lining of the intestines the cœlomic side of the epithelial cell rarely suffers any change. The luminal side undergoes the following changes: the thickened ends of the fibres disappear; the alveolar structure at the same time becomes concealed by a fluid substance which precipitates in killing fluids in the form of fine granules. The new chitin is probably formed by some process of hardening this substance. After the new lining begins to appear, delicate fibres are seen on the luminal side of the cell. The strength of the palisade on the luminal side varies directly with the time from the last moult.

8. The land isopods after a period of starvation will eat various kinds of pure foods.

9. Starving for a considerable time (three weeks) produces the following changes in the intestinal cells: the interalveolar substance is reduced in quantity; the cytoplasm may withdraw from the luminal side, leaving elongated spaces between the fibres; vacuolations may appear elsewhere in the cytoplasm.

10. After feeding a starved animal for ten minutes on finely chopped raw beef, before any food is visible in the cells, definite masses of fine granules appear about the nucleus. Some of these at least come from the nucleus. They increase in number up to thirty hours after feeding. We have designated this granular substance as an intracellular ferment.

11. Eight hours after feeding a starved animal with finely chopped raw beef, albumose appears in the intestinal cells. The

course of the albumose through the cells is uninfluenced by the cell structure, except in a purely mechanical way. In traversing the cell it behaves independently of the cytoplasmic structure. Albumose may accumulate on the cœlomic side of the cell from sixteen hours onward after feeding. Judging by the size of the granules formed by precipitation with killing fluids, albumose may exist in the cell in as great as a 10 per cent. solution. Albumose has not been found in the cœlomic fluid. It is probable that the intracellular ferment is concerned in the change of food from the albumose stage to a later stage of the hydrolisis (peptone) or to a stage in the inverse process toward albumen.

12. The cells of the typhlosole absorb soluble foods. The primary purpose of the structure, however, is to provide channels through which the secretion of the hepatopancreas may flow, unobstructed by solid food, to the median portion of the intestine.

13. Dextrose is found in the intestines of animals which have been starved, fed on potato starch, then killed in twenty-four hours from the time of feeding.

14. Microscopical study of the absorption of fats indicates: (*a*) That this class of foods is hydrolized by the digestive secretion of the heatopancreas; (*b*) that they are absorbed in the form of cleavage products, and (*c*) are at least partially synthesized into neutral fats under the influence of ferment action inside the cell; (*d*) they leave the cell not as discrete fat particles, but probably in the form of cleavage products; (*e*) they appear in the blood coagulum and in the blood corpuscles as neutral fats, reducing osmic acid and not staining with acid-fuchsine.

15. The hepatopancreas contains but one kind of secreting cells. In a young stage these cells contain zymogen granules in nascent condition, densely massed about the nuclei; as the cells mature the zymogen granules take up from the cytoplasm fatty substance, whereby they become larger, looser in structure, more soluble in many fixing fluids and more receptive of certain stains. The ferment thus matured is set free into the lumen by (*a*) fragmentation of the cell, (*b*) dissolution of the cell, (*c*) evacuation from the cell.

16. The secretion of the hepatopancreas thus elaborated contains ferments which act on the three classes of foods—proteids, carbohydrates and fats.

TABLE I.

No.	Genus.	Days starved.	No. of hrs. after feeding.	Fixation.	Secretion of Hepatopancreas.		Food in Cells of "Mid-gut."	
					In Lumen.	Zymogen in Cells.	Anterior Portion.	Median Portion.
1	P[22]	Fresh		Hermann's fluid[24] injected, 24 hrs.	Abundant.	All stages preserved. Small yellow to large black.	Food entering cells. Few gran. in various parts.	Gran. (albumose) on cœl. side of nucleus. (Fig. 9.)
2	P	Fresh		Hermann, 24 hrs.	Abund. Many cells recently disch.	Same as above.	No food distinguishable.	Gran. (albumose) luminal to cœl. side.
3	O[23]	14		Sublimate-acetic,[25] 3 hrs.	None in ant. Much in post.	Only in young cells.	Small gran. in masses.	Same scattered.
4	P	24	4	Hermann, 20 hrs.	Abundant. Cells in one tube all collapsed.		Few gran. (albumose) entering.	No. gran. (albumose).
5	P	24	4	Hermann (intestine injected), 20 hrs.	Abund. Luminal end of cells empty.	Young cells and in base of discharging cells.		Gran. chiefly on luminal side.
6	P	24	4	Hermann (intestine cut), 20 hrs.	Abund. Luminal end of cells empty.	Same as above.	Small gran. in masses.	
7	P	11	4	Platinic chloride-osmic acid mixture,[26] 15 hrs.			Small gran. in masses about nucleus. (Fig. 10, *B.*)	No free food distinguishable.

[22] *Porcellio.* [23] *Oniscus.* [24] For formulæ not given in the table, see Lee's *Vade Mecum*, 5th Ed. [25] Saturated aqueous solution of $HgCl_2$ with 2% acetic acid. [26] Hermann's fluid without acetic acid.

8	P	7	8	Platinic chloride osmic acid, 17 hrs.			Gran. (albumose) in masses of small granules. (Fig. 11, *A*).	No free food distinguishable.
9	P	14	9	Sublimate-acetic, 30 min.	Abund. Passing into int. Many cells disch. and l. end empty.	In young cells and up to nucl. in mature cells.	Few gran. (albumose) in streams.	Very little free food.
10	P	11	12	Platinic chloride-osmic acid, 15 hrs.	Abund. Passing into int. Many cells discharging.	In young cells and in mature cells to free end.	Small gran. in masses about nucleus.	Very few gran. (albumose).
11	O	2	12	Sublimate-acetic, 10 hrs.	Abund. Passing into int. Discharging cells in all parts.	In young cells.	Few gran. (albumose).	Gran. (albumose) on cœl. side of nucl.
12	O	21	16	Picro-acetic (Lee), 2 hrs.	In anterior end of intestine.		Small gran. about nucleus and in masses.	No free food distinguishable.
13	O	21	16	Sublimate-acetic, 30 min.	Abund. Passing into int. Cells all discharged. [Green.] [27]	In young cells.	*Moulting*. No signs of food.	Small yellowish gran. abundant on cœl. side of nucl.
14	O	21	16	Zenker's fluid, 2 hrs.	Abund. Passing into int. [Red.]	In young and mature cells. Cells gorged with blue-staining substance.	Gran. (albumose) abundant.	Large gran. (albumose) on cœlomic side of nucleus.

[27] Color after staining with Biondi-Heidenhain.

TABLE I (*continued*).

No.	Genus.	Days starved.	No. of hrs. after feeding.	Fixation.	Secretion of Hepatopancreas.		Food in Cells of "Mid-gut."	
					In Lumen.	Zymogen in Cells.	Anterior Portion.	Median Portion.
15	O	21	16	Formalin-alcohol,[28] 2 hrs.	Abundant. Passing into int. [Red.]	In young and mature cells. (Yellow.)	Small gran. in masses (yellowish).	Gran. on cœl. side of nucleus.
16	O	21	16	Picro-formalin, 2 hrs.	Abund. Passing into int. [Red.]	Yellow in young cells. Staining red in B.-H.[30] in mature	Small gran. in masses (yellowish).	Gran. on cœl. side in few cells.
17	O	21	16	Platinic chloride-osmic acid, 24 hrs.	Abund. Passing into int. Many cells discharging.	Brown to black. | young | mature	Small granules scattered.	Gran. (albumose) on cœl. side.
18	O	21	16	Picro acetic (Conklin),[29] 2 hrs.	Passing into intestine.	Young and mature, clear yellow.	Small gran. in masses about nucleus.	Yellow gran. abundant, esp. on cœl. side of nucleus.
19	O	21	16	Hermann, 24 hrs.	Abund. Passing into int. Many cells just discharged.	Brown to black, both young and mature cells.	Gran. (albumose) abundant.	Gran. (albumose) on cœl. side.
20	O	21	16	Flemming (Strong), 24 hrs.	None in ant. portion of tube.	Smaller gran. not preserved. Large glob. in mature, black.	Small gran. in masses. (Fig. 10, *A*.)	Gran. in streams and on cœl. side.

[28] 5% formalin, 1 pt.; 95% alcohol, 2 pts. [29] Half saturated solution of picric acid in 50% alcohol, with 2% acetic acid. [30] Biondi-Heidenhain.

21	P	8	18	Platinic chloride-osmic acid, 24 hrs.	Abund. Passing into int. Cells in all stages of discharging.	Brown in young to black in mature cells.	Gran. (albumose) in streams. (Fig. 11, *B.*)	No free food.
22	O	21	20	Sublimate-acetic, 100 min.	Abund. Passing into int. Cells med. height. [Red.]	Only in young cells.	Small gran. in masses and scattered (yellowish).	Same gran. on cœl. side of nucl.
23	P	14	24	Hermann, 24 hrs.		Brown in young, black in mature cells.		Gran. albumose in streams. Plate XVI, fig.1
24	P	5	24	95% alcohol, 24 hrs.	Abundant.	Not preserved.	No albumose.	No albumose. Plate XVI, fig.2
25	P	8	24	Platinic chloride-osmic acid, 18 hrs.	Abundant. Cells discharging.	Brown in young, black in mature.	Small gran. in masses with large gran. (albumose).	Gran. (albumose) on cœl. side of nucleus.
26	P	14	26	Sublimate-acetic, 30 min.	Plentiful. None passing into intestine. Few cells discharging.	Young and mature, yellow.	No food found. Animal may have been about to moult.	
27	P	8	32	Platinic chloride-osmic acid, 24 hrs.	Abundant. Passing into int. Cells discharging.	Brown in young, black in mature.	Small gran. and large (albumose).	Gran. (albumose) on cœl. side.
28	O	2	32	Saturated sublimate, 2 hrs.	Quantity small in distal portion.	Not well preserved.	Small gran. in masses.	Gran. (albumose) on cœl. side.

TABLE I (*continued*).

No.	Genus.	Days starved.	No. of hrs. after feeding.	Fixation.	Secretion of Hepatopancreas.		Food in Cells of "Mid-gut."	
					In Lumen.	Zymogen in Cells.	Anterior Portion.	Median Portion.
29	P	14	42	Picro-acetic (Lee), 2 hrs.	Abundant. Most of cells disch. Some discharging.	In young cells.	Gran. in masses (albumose).	Gran. (albumose) on cœl. side of nucl.
30	O	14	42	Sublimate-acetic, 30 min.	None in prox. end of tube. Many cells discharged.	In young cells: in mature at proximal end.	*Moulting.* Small gran. on c. side.	*Moulting.* Small gran. on cœl. side.
31	O	14	42	Saturated sublimate, 2 hrs.	Abund. Passing into int.	Not much preserved.	Gran. (albumose) abundant.	Food not definite, gran. on cœl. side.
32	O	14	48	Formalin-alcohol, 1 hr.	Abund. in prox. end. [Red.]	None preserved.	Gran. in masses.	Gran. on cœl. side.
33	O	14	48	Picro-formalin, 50 min.			Very large gran. (albumose) with masses of small gran.	Gran. (albumose) on cœl. side.
34	O	14	48	Platinic chloride-osmic acid, 48 hrs.	Abundant. Passing into int. Many cells discharging.	Brown in young, black in mature. All glob. preserved.	Very large gran. (albumose) with masses of small gran. on c. side. Plate XVI, fig. 3.	Gran. (albumose) very abund. all through the cell.

35	P	12	50	Hermann, 48 hrs.	Abundant. Cells med. height in prox., discharged in distal.	Brown in young, red in mature. (Acid Fuchsine.)	Gran. albumose passing around nucl., also small black gran.	Gran. albumose very abundant, also small black gran.
36	P	21	50	95% alcohol, 12 hrs.	Abundant.	None preserved.	No albumose. Plate XVI, fig. 4.	No albumose.
37	P	20	74	Hermann, 12 hrs.		Brown in all cells.	Few gran. albumose.	Gran. albumose also small black gran. very abundant.
38	P	19	74	95% alcohol, 12 hrs.	Abundant.	None preserved.	No albumose.	No albumose.
39	P	14	90	Platinic chloride-osmic acid, 48 hrs.	Much in prox. end. Less in distal. Some cells discharging.	Preserved only in young cells.	Few gran. (albumose).	Few gran. (albumose).
40	P	14	90	Sublimate-acetic, 35 min.	Much. Young cells elongating.	Very abundant in young cells.	Small granules in masses, especially about nucleus.	No free food.
41	O	14	90	Sublimate-acetic, 40 min.	Little in prox. Much in distal. Some young cells seem to be discharging.	None preserved.	Flocculent gran. masses staining with Bismarck brown.	Same.

TABLE I (*continued*).

No.	Genus.	Days starved.	No. of hrs. after feeding.	Fixation.	Secretion of Hepatopancreas.		Food in Cells of "Mid-gut."	
					In Lumen.	Zymogen in Cells.	Anterior Portion.	Median Portion.
42	P	14	98	Sublimate-acetic, 40 min.	Little in prox. Much in distal.	Abundant in young only.	Flocculent gran. masses abundant.	No free food. Empty spaces in cell.
43	O	14	100	Picro-acetic (Conklin), 4 hrs.	Little in all parts of tube.	Very little preserved.	Gran. in masses about nucleus (yellowish).	Same gran. on cœl. side of nucl.
44	O	14	120	Sublimate-acetic, 40 min.	Abundant throughout. Cells discharged.	Only in young cells, stains bluish. B.-H.	Small brownish gran. abundant. Large gran. (albumose) few.	Small, poorly staining gran. on cœl. side.
45	P	3	120	Hermann (intestine cut), 10 hrs.	Abundant. Most cells discharged.	Very abundant in young cells elongating.	Small gran. in masses.	Food not definite. Gran. on cœl. side
46	O	18	7 days on meat	Sublimate-acetic, 50 min.	Much. Cells gorged with blue-staining mass.	Few gran. yellow. Many in mature cells stain red, B.-H.	Gran. (albumose) very abundant.	Some very large gran. (albumose).

TABLE II.

	Precipitated by	Further Treatment.	Result.
(1) 1% Hemialbumose.	5% $HgCl_2$	Washed in alcohol, stained in 1% aqueous Acid Fuchsin, 10 min.	All coagulum, no granules.
(2) 1% Deuteroalbumose.	5% $HgCl_2$	Dried from 95% alcohol, st. in 7% aq. Acid Fuch. 15 min.	Few very large granules in dense coag.
(3) 2% Peptone.	5% $HgCl_2$	Dried from 95% alcohol, st. in 7% Acid Fuch. in aniline water.	All very sharp granules of uniform size.
(4) 2% Peptone.	Flemming.	Washed 6 hrs. in running water, st. 7% Acid Fuch. in aniline water 10 min.	All sharp granules uniform size.
(5) 2.5% Peptone.	1% Chromic Acid.	Washed 6 hrs. in running water, st. 7% Acid Fuch. in aniline water 15 min.	Same as above, smaller than from 5% Pep. (q. v.), fig. 13.
(6) 5% Peptone.	1% Chromic.	Same as above.	All granules: not uniform. Larger than from 2.5% Pep. (q. v.), fig. 13.
(7) 5% Peptone.	Hermann's fluid without Acetic.	Same as above.	All granules uniform size, fig. 5, Plate XVI.
(8) 5% Peptone.	95% Alcohol.	Washed 1 hr. in running water (from 50% alcohol), drying, the granules melt away: may be re-precipitated.	Granules like above all dissolved.
(9) 5% Peptone.	5% (HgI_2 KI)	Washed in running water 4 hrs.	Granules like above slowly dissolve.
(10) ½% Serumalbumen. ½% Deuteroalbumose.	2% Osmic 1 pt. 2% Platinic-Cl. 3 pts.	Washed 6 hrs. in running water, st. in Iron-Hæmatoxylin.	Few well defined granules in dense coagulum.
(11) ½% Serumalbumen. ½% Deuteroalbumose.	Hermann.	Washed 6 hrs. in running water, st. in 7% Acid Fuch. in aniline water.	Granules imbedded in coagulum, fig. 6, Plate XVI.
(12) 2% Serumalbumen.	1% $HgCl_2$	Washed 6 hrs. in running water, then impregnated with [1% D., next line].	Coagulum in which were granules as in fig. 7, Plate XVI.
1% Deuteroalbumose.	Flemming.	Washed 24 hrs. in running water, st. in 7% Acid Fuch. in aniline water 20 min.	

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EXPLANATION OF PLATE XVI.

All figures drawn by the aid of the camera lucida × *600.*

Fig. 1.—Median cell from intestine of *Porcellio scaber*, fed with raw beef; intestine fixed after 24 hrs. in Hermann's fluid (24 hrs.) and washed in water for 24 hrs. Sections stained in 15 per cent. acid-fuchsine in aniline water. *Alb. Gr.*, albumose granules.

Fig. 2.—Median cell from intestine of *Porcellio scaber*, fed with raw beef; intestine fixed after 24 hrs. in 95 per cent. alcohol (24 hrs.), washed in lower grades and finally in water 15 hrs. Sections stained in 15 per cent. acid-fuchsine in aniline water. Albumose granules washed out. Nucleus shows effects of unilateral penetration of the fixing fluid.

Fig. 3.—Median cell from intestine of *Porcellio scaber*, fed with raw beef; intestine fixed after 50 hrs. in Hermann's fluid (24 hrs.), and washed in water 24 hrs. *Alb. Gr.*, albumose granules.

Fig. 4.—Median cell from intestine of *Porcellio scaber*, fed with raw beef; intestine fixed after 50 hrs. in 95 per cent. alcohol (24 hrs.), and washed in water 15 hrs. Albumose granules washed out. The nucleus is distorted by the fixation. The mass which occupied the concavity of the nucleus previous to washing may have contained albumose. Compare fig. 3.

Fig. 5.—Two per cent. peptone (depur. sicc. aus Fibrine, Grübler) precipitated on cover-glass with Flemming's strong fluid. Washed in running water 6 hrs. Stained in 7 per cent. acid-fuchsine.

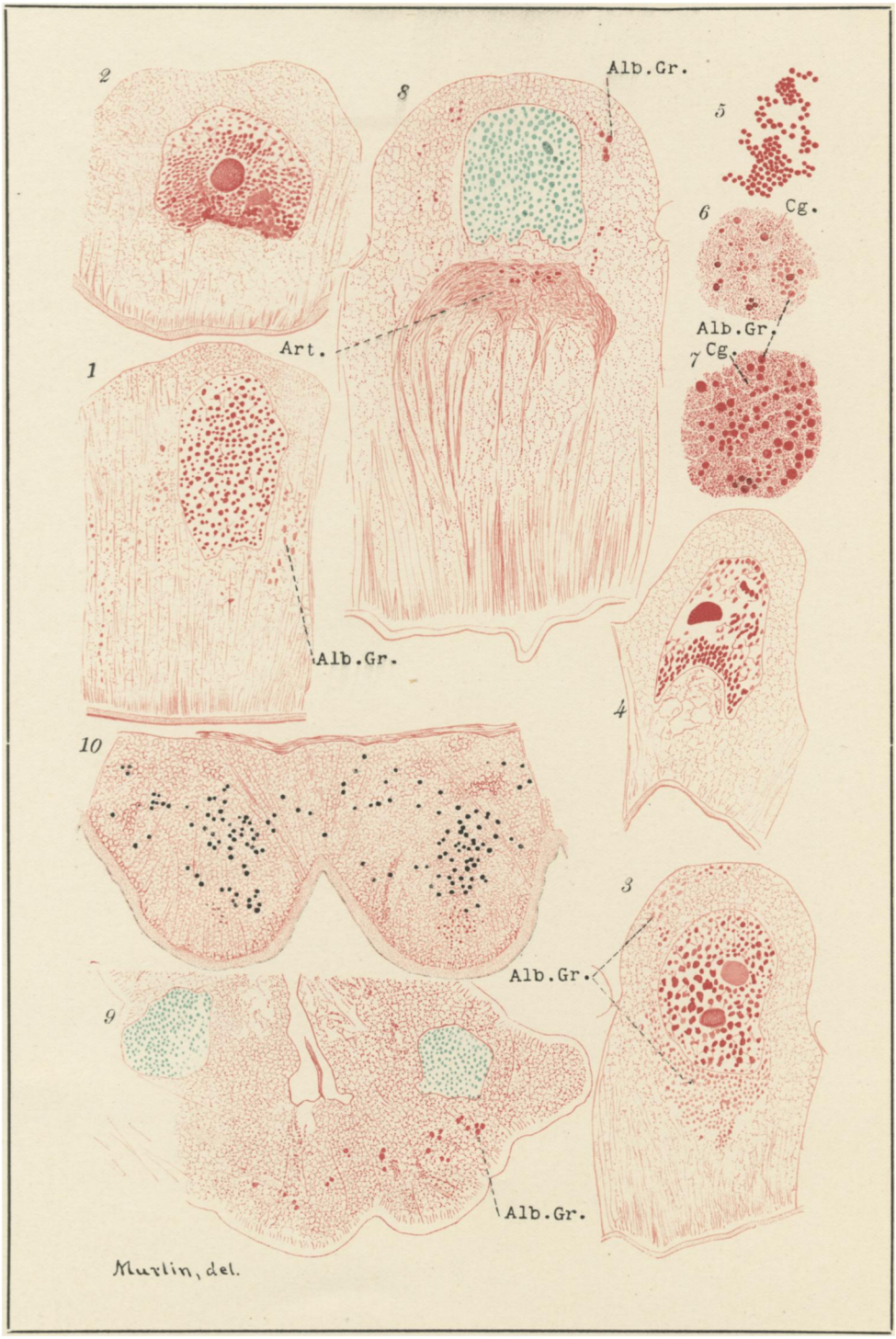
Fig. 6.—Two per cent. serumalbumen and 2 per cent. deuteroalbumose (Grübler) (equal parts) precipitated on cover-glass by Hermann's fluid, washed in running water 6 hrs., and stained in 7 per cent. acid-fuchsine in aniline water. *Cg.*, coagulum of albumen; *Alb. Gr.*, albumose granules.

Fig. 7.—Two per cent. serumalbumen precipitated by 1 per cent. $HgCl_2$ and washed in running water 6 hrs.; then impregnated with 1 per cent. Deuteroalbumose 2 hrs. and again precipitated by Flemming's fluid (24 hrs.), and stained in 7 per cent. acid-fuchsine in aniline water. *Cg.*, coagulum of albumen; *Alb. Gr.*, albumose granules.

Fig. 8.—Median cell from "mid-gut" of *Oniscus asellus*, fed with raw beef; intestine fixed after 20 hrs. in sublimate-acetic. Sections stained in Biondi-Hiedenhain. *Art.*, artifact caused by unilateral penetration of the fixing fluid (see p. 305); *Alb. Gr.*, albumose granules.

Fig. 9.—Median portion of the typhlosole from intestine of *Porcellio spinicornis*, fed with raw beef; intestine fixed after 24 hrs. in sublimate-acetic. *Alb. Gr.*, albumose granules.

Fig. 10.—Two anterior cells from intestine of *Porcellio scaber*, fed with olive oil and fixed after 24 hrs. in 1 per cent. platinic chloride 15 pts., 1 per cent. osmic acid 4 pts. Sections stained in 10 per cent. acid-fuchsine. All black granules are fat.



MURLIN. DIGESTIVE SYSTEM OF ISOPODS.